MEDICAL LABORATORY METHODS

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MEDICAL LABORATORY METHODS AND TESTS

BY

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LONDON
BAILLIÈRE, TINDALL AND COX
8, HENRIETTA STREET, COVENT GARDEN
1904

PREFACE

THIS volume has been written in response to repeated complaints that there was no *small* book dealing with the chemical and microscopical tests and investigations which are most useful to medical men. The object has been to detail the commoner methods, pointing out the conclusions which may be drawn from the various tests, but laying stress upon the fallacies to which each is liable. The book deals, not with the examination of patients, but with that of fluids or substances obtained from them, and bedside methods have been excluded. It is intended to be a small handbook for the medical laboratory.

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March 19, 1904.

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CHAPTER I.

EXAMINATION OF THE URINE.

I. GENERAL CHARACTERISTICS OF URINE.

THE *amount* passed in twenty-four hours is, roughly, 1,500 c.c., or 50 ounces; but varies within wide limits. Free perspiration diminishes it, as in summer and after exercise; it is increased in winter and after copious drinking. As passed, it is usually quite clear. On standing, a faint cloud of 'mucus' often forms, especially in women. A dense pink precipitate of urates is common in summer urine; a white precipitate of earthy phosphates is not unusual in the 'alkaline tide' which follows a full meal. Some common pathological changes in amount are:

Increase in—	*Decrease in—*
Granular kidney, 80 to 100 ounces.	Fevers, such as pneumonia—*e.g.*, 25 ounces.
Diabetes mellitus, 100 to 200 ounces.	Acute nephritis—*e.g.*, 10 ounces or even none at all.
Diabetes insipidus, 100 to 300 ounces.	Heart failure—*e.g.*, 20 ounces, 10 ounces, or less.
Hysteria, After epileptic fit, After head injury, Recovery from anasarca, — Up to 100 ounces.	After catheterization it may be entirely suppressed.

The *specific gravity* is determined by the urinometer, the scale being read at the point where the graduated stem leaves the fluid. If there be insufficient urine to float the urinometer, add an equal bulk of distilled water; multiply the last two figures of the specific gravity of the mixture by 2 to give that of the original urine. In health it varies from 1005 after much drinking to 1030 after profuse perspiration.

Pathological changes in specific gravity :

Unduly low :	*Unduly high :*
Diabetes insipidus, 1002 or 1004.	Heart failure, 1030 to 1035.
Hysteria, 1004.	Acute fevers, 1030 to 1035.
Post-epileptic, 1004.	Diabetes mellitus, 1035 to 1045.
Granular kidney, 1008 to 1012.	

The *reaction* is determined with litmus paper, 'acid' urine turning it red, 'alkaline' blue; when both red and blue litmus papers are turned purple the reaction is termed 'amphoteric.' The acidity is due to acid sodium phosphate; an alkaline tide follows the big meal of the day, owing to the increased acid secretion in the stomach. The urine is more acid in those who eat meat; in vegetarians it may be alkaline. On standing, the acidity at first increases, then diminishes, and is changed to alkalinity by conversion of urea into ammonia.

Pathological changes in reaction :

Increased Acidity.	*Alkalinity.*
In acute fevers—*e.g.*, pneumonia, acute rheumatism.	Suppuration in the bladder.
In the uric acid diathesis.	

The *colour* of normal urine, due to urochrome, varies

from pale straw-yellow (low specific gravity) to dark sherry (high specific gravity).

Pathological changes in colour (for tests, see pp. 20, 31, 34-39):

Pallor: in diabetes mellitus, diabetes insipidus, granular kidney, hysteria, epilepsy, head injury, excessive drinking.

Blood: may make the urine bright red, dark red, port-wine colour; brown, brown-black, or black.

Red-brown: after rhubarb, senna, chrysophanic acid.

Greenish yellow to greenish black: bile pigments; phenyl compounds, such as carbolic acid, salol, resorcin, guaiacol, creosote, alkapton, naphthaline, indican; melanin.

Bright yellow: after santonin.

Dichroïc: urobilin, red by transmitted, green by reflected light.

Blue: after the administration of methylene blue.

The *smell* of normal urine is 'aromatic,' but becomes ammoniacal on standing.

Pathological changes in smell:

Ammoniacal: in cystitis.

Fæcal: from contamination with *Bacillus coli communis.*

Like violets: after the administration of turpentine.

Sweet: in diabetes mellitus.

After eating asparagus, the urine has a very characteristic odour.

II. URINE DEPOSITS.

A. Crystalline Deposits :

1. *Calcium Oxalate.*—'Envelope' crystals under a low power ; really octahedra, but appearing as squares with a diagonal cross (Fig. 1).

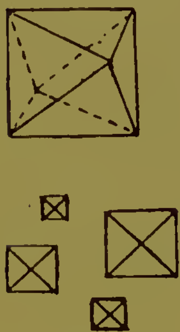
Fig. 1. — Calcium Oxalate.

Importance. — They occur in many healthy urines, whether slightly acid, amphoteric, or slightly alkaline ; in very acid urines, oxalates remain in solution.

In cases of suspected stone in the kidney, envelope crystals point to a 'mulberry' calculus.

They are particularly abundant after eating rhubarb ; and, to a less extent, after tomatoes, cauliflower, and other vegetables.

When so persistent as to constitute 'oxaluria,' they are a sign of acid dyspepsia.

2. *Uric Acid.*—The crystals are always coloured by the urochrome, and have a variety of shapes, the commoner being 'whetstones,' 'rosettes,' 'bundles,' and 'dumb - bells' (Fig. 2, 1-4).

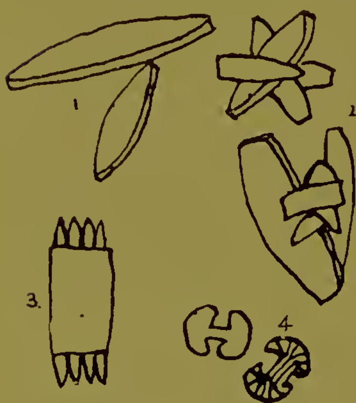

Fig. 2.—Uric Acid.

Importance. — They occur in acid urine as a deposit of cayenne-pepper-like grains. They indicate hyperacidity of the urine. The con-

dition is not necessarily gouty, nor is the uric acid necessarily in excess. The hyperacidity may cause irritability of the bladder or urethritis, and the crystals may form a calculus. Treatment should be directed to diminishing the acidity of the urine in order to keep the uric acid in solution.

3. *Triple Phosphate*, or ammonio-magnesium phosphate, $NH_4.Mg.PO_4$: described as 'coffin-lid' or 'knife-rester' crystals (Fig. 3).

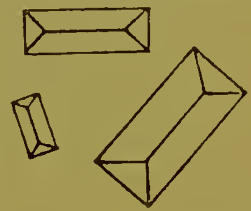

FIG. 3.—Triple Phosphate.

Importance.—They occur in urines which contain ammonia. To the naked eye the deposit is heavy, opaque white, and may be mistaken for pus. The ammoniacal change may have occurred since the urine was passed; the crystals then have no pathological import. If found in fresh urine, they indicate cystitis, or other cause of decomposition within the urinary tract.

4. *Calcium, or 'Earthy,' Phosphate.*—This is usually amorphous; rarely it assumes the form of colourless elongated prisms, occurring singly, or in starlike clusters of 'stellar phosphate' (Fig. 4).

FIG. 4.—Stellar Phosphate.

Importance.—They occur in faintly acid, neutral, or alkaline urines; they are 'earthy' as distinct from alkaline or 'triple' phosphate, and do not indicate disease.

5. *Sodium Urate and Ammonium Urate.*—'Thorn-apple' crystals, seen under the high power as small spheres with numerous short spines (Fig. 5).

Fig. 5.—Thorn-apple Crystals.

Importance. — Sodium urate crystals occur in the urine of newly-born infants. 'Thorn-apple' crystals in adults are due to ammonium urate, in alkaline urines, in association with triple phosphate. They indicate decomposition, either after the urine has been passed, or, in cases of cystitis, within the urinary tract.

6. *Tyrosine.*—Sheaves of colourless needles (Fig. 6), to be distinguished from phenyl-glucazone crystals by (1) their smaller size, (2) their being colourless.

Fig. 6.—Tyrosine.

Importance.—They are rare; are usually associated with leucin; and are most typically seen in cases of acute yellow atrophy of the liver. They may be found in other affections of the liver also—*e.g.*, in phosphorus poisoning.

7. *Leucin.* — Pale yellow spherical masses, often concentrically striated (Fig. 7).

Fig. 7.—Leucin.

Importance.—The same as that of tyrosine. Leucin and tyrosine are amido compounds which, in health, should be converted into urea by the liver.

8. *Cystin.*—Hexagonal plates in acid urine (Fig. 8).

Importance. — Cystin is a rare constituent of urine; but, if present in one member of a family, is liable to occur in others also. It does not indicate ill-health, though it has given rise to calculi.

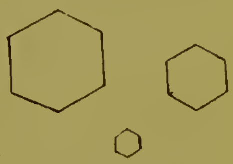

FIG. 8.—Cystin.

B. AMORPHOUS NON-ORGANIZED DEPOSITS:

1. *Urates.*—Those of sodium and potassium occur in concentrated acid urines, that of ammonium in alkaline. They have great affinity for uroerythrin, which gives the deposit a characteristic pink colour. Under the microscope, granular particles are seen, running together into moss-like clumps. Some of the ammonium urate may at the same time be in the form of 'thorn-apple' crystals. In the absence of uroerythrin urate deposits are white like phosphates.

To confirm, warm the urine to body temperature; urates redissolve, whereas phosphates increase. In case albumin be present, fill 3 inches of a test-tube with the urine and its deposit; warm the upper 2 inches gently. Urates will clear up. Boil the top 1 inch; albumin, if present, will form a cloud, so that in the top 1 inch there is a cloud of albumin; in the middle 1 inch clear urine in which urates have redissolved at body-heat; at the bottom the original deposit of urates (Fig. 9).

FIG. 9.

Importance of Urates.—They indicate a concentrated urine, but afford no clue to the cause of concentration; it may be physiological, or may

be due to pathological conditions, such as acute fevers, heart failure, or excessive loss of fluid from the skin or bowel.

2. *Earthy Phosphates—i.e., Phosphates of Calcium and Magnesium.*—To the naked eye the deposit is white and flocculent, and may be confused with thick mucus, pus, alkaline phosphates, or colourless urates. When blood is present the methæmoglobin tinges the phosphates a dirty brown. The urine may be alkaline, amphoteric, or just acid. Under the high power granular particles not unlike those of amorphous urates are seen, with an occasional 'stellar phosphate' crystal.

To some of the deposit in a test-tube add acetic acid; phosphates will redissolve; pus or urates will remain unaltered; mucus will become more marked. Do not test with nitric acid; for this, though it would redissolve the phosphates, would at the same time precipitate any albumin present.

Phosphates, being less soluble in hot than in cold urines, often form a cloud on boiling; this clears up at once on the addition of acetic acid.

Importance of Earthy Phosphate Deposit.—It has no pathological significance; it indicates that the urine is not very acid.

3. *Starch Grains.*—These appear under the microscope as round or oval bodies, with crescentic markings. They may be detected by their blue colour on adding iodine.

Importance.—They are adventitious, from dusting-powders; but may be mistaken for leucin until the iodine test has been applied.

C. ORGANIZED SEDIMENTS :

For the microscopical examination of these, the urine should be centrifugalized.

1. *Epithelium :*

(*a*) *Squamous Cells.*—Of large size, with a small central nucleus. These occur singly (Fig. 10), or in groups of two or three, or in sheets of twenty or more.

FIG. 10.—Squamous Cells.

Importance.—They are derived from the vagina; if very numerous, they indicate vaginitis and catarrh ; in smaller numbers they have no significance.

(*b*) *Bladder Cells.*—Though variable in appearance, these are of two types. Those from the deep layer in the bladder are oval at one end, and at the other taper into a long or short tail (Fig. 11, B); the superficial cells are convex on their free surface, with concavities on their under side, into which the oval cells of the deep layer have fitted (Fig. 11, A).

A B

FIG. 11.—Bladder Cells.
A, superficial ; B, deep.

Importance.—They are not pathological unless in large numbers, when they suggest vesical catarrh or cystitis.

(*c*) *Renal Cells.*—These are two or three times the size of leucocytes ; and, when fresh, have a polygonal outline and central nucleus (Fig. 12, A). More fre-

quently they have undergone fatty degeneration, and appear spherical, and opaque with fine granules, amongst which the nucleus is no longer distinguishable (Fig. 12, B).

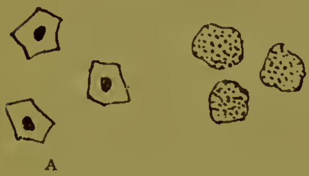

Fig. 12.—Renal cells. A, fresh; B, fatty.

Importance. — They seldom occur in health, but indicate a catarrhal condition of the kidney. They may be very numerous in acute and chronic tubal nephritis, but occur also in renal congestion from heart failure; after infarction; with renal calculus, tuberculous kidney, and with growth. In the last, small masses of similar cells detached from the growth may assist the diagnosis.

2. *Leucocytes.*—If fresh, these appear exactly like those of the blood, and are mostly polymorphonucleated; if the urine has stood, they become granular and fatty, but are distinguished from fatty renal cells by their smaller size (Fig. 13). Acetic acid renders the nucleus more distinct.

Fig. 13.—Leucocytes.

Importance.—Small numbers indicate irritation within the urinary tract. They occur in Bright's disease, urethritis, vesical catarrh, and in association with blood in the urine. Large numbers indicate purulent inflammation, such as gonorrhœal urethritis, gleet, prostatitis, cystitis, renal growth, tubercle or calculus, or suppurative nephritis.

3. *Red Blood Corpuscles.*—These often look like those

of fresh blood ; in very dilute urines they may be laked, and appear as 'ghosts'; in concentrated urines they may be crenated, and variously crumpled.

Importance.—In a woman they may be derived from menstrual blood. Otherwise, their presence is the best test of hæmaturia. They may be derived from the kidney, the ureter, the bladder, the prostate, the urethra, a caruncle, and the microscope affords no evidence of their source. Apart from menstruation, they are always pathological.

FIG. 14.—Renal Casts.

4. *Casts.*—There are the following varieties (Fig. 14):

i. Epithelial casts, in which outlines of renal cells are seen.

ii. Blood casts, composed of red corpuscles.

iii. Leucocyte casts, consisting of white corpuscles, which are distinguished from renal cells by their smaller size.

iv. Fatty casts, not very different to granular

casts, but containing here and there small highly refractile specks and globules of fat.

v. Granular casts having no cell elements, and a typical granular appearance.

vi. Hyaline and waxy casts, seen only in outline, the cast itself having no internal structure.

Many become broken and unrecognisable; some appear of different structure in different parts—*e.g.*, granular at one end, epithelial at the other; and there is a tendency for crystals, pus corpuscles, fungi to become attached to them and obscure their outline.

Importance. — It would seem probable that epithelial, fatty, granular, and hyaline casts are progressive downward changes in casts derived from the same source—namely, the renal tubule cells—whereas blood and leucocyte casts depend on exudation from vessels. An occasional granular or hyaline cast is to be found in healthy urine that has been centrifugalized. Small numbers may be found when the kidney is passively congested, from failure of the right side of the heart. Blood casts may occur in blood diseases, and with other causes of hæmorrhage within the kidney substance, such as infarction or venous thrombosis. But the presence of many casts points strongly to nephritis. The more acute the nephritis, the more rapidly will the casts be formed, and the less change will they have undergone; the vascular changes will be the more marked also, with exudation of red and white corpuscles. Therefore epithelial, blood, and leucocyte casts indicate acute renal inflammation;

as the latter subsides, blood and leucocyte casts disappear, epithelial casts grow fewer, and the fatty casts increase; in chronic nephritis, hardly any epithelial casts are seen, fatty casts are few, granular and hyaline preponderate.

Waxy casts are not essentially different to hyaline; they are more refractile and more easily seen, but have no significance that hyaline casts have not.

5. *Spermatozoa.*—These seldom give rise to difficulty under a high power each is seen to have an ovoid head about two-thirds the size of a red corpuscle, and a thread-like tail, usually broken off short, but if complete, about as long as eight red corpuscles (Fig 15).

FIG. 15.—Spermatozoa.

Importance.—Usually none; their presence may account for a trace of albumin in an otherwise healthy urine.

6. *Bacteria.*—In warm weather normal urine soon swarms with the micrococcus ureæ, which converts urea into ammonium carbonate. Associated with it may be hosts of non-pathogenic bacilli.

Importance.—None, except that if not recognised they may be mistaken for organisms which cause disease.

The pathogenic organisms which may be found are:

Staphylococci.	Tubercle bacilli.
Streptococci.	Bacilli coli communis.
Gonococci.	Typhoid bacilli.

In examining for any of these, first centrifugalize

the urine well. Then make a cover-slip film. In the absence of albumin the bacteria will not remain fixed to the glass. To obviate this, albuminize the cover-slips as follows: Mix some fresh white of egg with nine times its bulk of water, and filter it; smear the filtered egg-white upon one surface of the cover-slip, and allow it to dry unexposed to dust. Slips albuminized in this way can be kept for future use. To find out which surface is albuminized, breathe on it; the unalbuminized surface becomes opaque from condensed moisture, the other remains transparent. Smear the urine deposit thickly on this surface; allow it to dry; fix the film by passing it through a Bunsen flame five times.

For *tubercle bacilli* stain by the Ziehl-Neelsen method (p. 88).

> *Importance.*—If present, they indicate a tuberculous lesion of the kidney, bladder, epididymis, or seminal vesicles. If absent, even after careful search, a tuberculous lesion is not excluded; more often than not the tubercle bacilli cannot be found.
>
> A source of fallacy is the *smegma bacillus*, which also stains by the Ziehl-Neelsen method; it will not retain the stain, however, if the film be first immersed for ten minutes in warm caustic soda solution containing 5 per cent. of alcohol, whereas tubercle bacilli will still stain as before.

For *gonococcus*, *Bacillus coli communis*, *typhoid bacillus*, *streptococcus*, and *staphylococcus*, stain with carbol methylene blue or carbol fuchsin (p. 95). Staphylococci and streptococci may also be stained by Gram's method (p. 96); the others not.

Importance.—All these organisms are pathogenic, and any of them may cause cystitis. Gonococci more often come from the urethra; typhoid bacillus is sometimes excreted in what appears to be healthy urine long after convalescence from enteric fever, but cannot be distinguished by the microscope from *Bacillus coli communis.*

7. *Yeast Fungi* (Fig. 59, p. 117) have no pathological significance; in warm weather they commonly occur, particularly in diabetic urine which has stood open to the air.

8. *Fat Globules.*—Highly refractile particles, readily stained black by osmic acid, and dissolved by ether.

Importance.—In temperate climates it is very rare to find fat in the urine, unless by accidental or intentional admixture of milk or oil—*e.g.*, after vomiting, or the passage of an oiled catheter. True chyluria results most commonly from infection with filaria in the tropics; the blood should be examined for this parasite (p. 78).

9. *Bilharzia Hæmatobia.*—This is the only parasite likely to affect the urine. The mature worm occupies the pelvic vessels; the eggs work their way through the walls of the latter, and reach the urine through the bladder wall, producing hæmaturia. Each worm is $\frac{1}{200}$ inch long, and half as broad; with the low power it resembles a melon seed, with a short curved spine at one end, or, in some cases, on one side (Fig. 16). The contained embryo

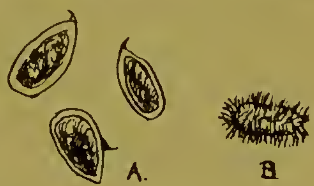

FIG. 16.—Bilharzia hæmatobia.
A, ova; B, ciliated embryo.

will not hatch in urine, but in clean tepid water it bursts its chitinous shell, and moves about by means of its many cilia.

Importance.—The parasite is acquired in some way from water, chiefly in Egypt and South Africa. Many cases occurred during the late war. The diagnosis of the cause of the hæmaturia depends on finding the ova.

10. *Prostatic Threads.*—If the urine be poured into a glass cylinder, these may be seen by the naked eye floating about as fine threads; being composed of mucus, they appear structureless under the low power. They may be mistaken for renal casts, but are much larger and less regular in outline.

Importance.—They point to previous gonorrhœa. In cases of multiple arthritis of uncertain origin, presence of prostatic threads supports a diagnosis of gonorrhœal rheumatism.

III. TESTS FOR SUBSTANCES IN SOLUTION.

1. Albumin.—Filter the urine if it be turbid; it is essential for the following tests that it should be clear.

Heller's Nitric Acid Test.—Pour 1 inch of non-fuming nitric acid into a test-tube; hold the latter slanting, and allow urine to flow gently from a pipette on to the surface of the acid. Watch the line of junction; an opaque white ring will appear at once if much albumin be present, more slowly with less. A brownish-red ring which often appears also is quite independent of

the presence of albumin, and results from the oxidation of indican by the nitric acid.

Fallacies:

(1) If fuming nitric acid be used, the urea will be decomposed, giving off bubbles of CO_2 and N_2. This will cause the urine to mix with the acid so that the line of junction is destroyed; no white ring will then appear, though albumin be present.

(2) Albumose will give a similar white ring; but it disappears on warming to reappear on cooling, whereas that due to albumin remains on warming.

(3) If the urine be concentrated, urea nitrate may form a white ring. Dilute the urine with an equal bulk of water, and repeat the test. Urea nitrate will now remain in solution.

(4) Uric acid, in concentrated urine, may form a similar ring; not, however, quite at the junction of the two fluids, but somewhat higher. It will not appear if the urine be first diluted.

(5) After the administration of drugs containing resins—*e.g.*, copaiba—a white ring appears with nitric acid; it is more diffuse than that of albumin. It is due to precipitation of the resin, which will redissolve on adding a little alcohol.

(6) Mucin may cause a faint cloud with nitric acid, but rather in the supernatant fluid than at the line of junction of the two.

Test by Heating.—Fill a test-tube three parts full of urine; note the reaction to litmus paper. If alkaline, just acidify with acetic acid; the object of this is to prevent the formation of uncoagulable alkali-albumin

on warming. Acetic acid is used because a stronger, such as hydrochloric, would form acid-albumin, which is likewise uncoagulable by heat. Boil the upper 1 inch thoroughly, and look through the test-tube at a dark background. If the boiled inch is as clear as the unboiled urine, no albumin is present; if it has become cloudy, add another drop of acetic acid. If the cloud disappears entirely, it is due to phosphates; if it be due to albumin it will remain.

Fallacies:

(1) If the urine be not acid, alkali-albumin may be formed, and no cloud appear, though albumin be present.

(2) The cloud may be due to phosphates; if so, it will clear up with acetic acid.

(3) Nucleo-albumin and mucin may cause a slight turbidity on boiling; they will be redissolved on adding a drop of nitric acid, whereas albumin would remain.

The heat test is the best for albumin, and has the fewest fallacies.

Potassium Ferrocyanide Test.—To 2 inches of urine in a test-tube add 10 drops of a 5 per cent. solution of potassium ferrocyanide; acidify strongly with acetic acid. If albumin be present, turbidity appears.

Fallacies:

(1) The test is not very delicate.

(2) Albumose gives a similar turbidity. Test with nitric acid; a white ring, disappearing with warmth, reappearing on cooling, indicates albumose.

(3) Nucleo-albumin also gives turbidity. Test

a fresh sample of urine with acetic acid without potassium ferrocyanide; turbidity indicates nucleo-albumin.

Picric Acid Test.—To 2 inches of saturated solution of picric acid in a test-tube add urine drop by drop from a pipette. Albumin forms a precipitate round each drop.

Fallacies :

(1) Albumoses and peptones also form a cloud. It disappears on warming, that of albumin does not.

(2) Quinine gives a similar cloud, which also disappears on warming.

Importance of Albumin.—The presence of albumin in the urine is seldom without significance, though far from always meaning Bright's disease. In women, it may be an accidental accompaniment at the menstrual period, or arise from vaginitis, or some uterine discharge; in men it may be temporary after seminal emission; and in boys a so-called 'functional' albuminuria is almost common about the time of puberty. With blood or pus albumin is always present. It is frequent with heart failure, and may result from any cause of low arterial blood-pressure. Abdominal tumours, ascites, and inferior vena cava thrombosis may cause albuminuria by obstructing the outflow from the renal veins. It is not uncommon in the severe anæmias, and accompanies lardaceous disease of the kidneys. Only when casts are present in proportion can Bright's disease be diagnosed with certainty.

2. Blood.

Guaiacum Test.—To 1 inch of urine in a test-tube add 1 drop of tincture of guaiacum; the resin forms a white precipitate. Pour on to the surface 1 inch of ozonic ether. If blood be present, it and the ozonic ether together oxidize the guaiacum, and a blue colour appears at the junction of the fluids.

Fallacies:

(1) Pus gives a similar colour, but it is more green than blue, and appears more slowly. Pus will give the colour with the guaiacum alone.

(2) Iodides in the urine give a similar blue colour, but it appears more slowly than with blood.

(3) Saliva in quantity also gives the blue colour; the fallacy may be avoided by testing a catheter specimen.

Heller's Test.—Phosphates have an affinity for blood pigment, and carry it down with them. Render some urine in a test-tube strongly alkaline with caustic soda, and boil it; if blood be present, the deposit of earthy phosphates is brownish-red.

Fallacies:

(1) Earthy phosphates may be deficient in the urine, and no deposit result. To obviate this, add 2 drops of calcium chloride solution.

(2) Certain drugs—rhubarb, senna, santonin—give a similar reaction.

Spectroscope Test.—Even a small pocket spectroscope is expensive, so that the test is little employed. When the microscope reveals red corpuscles, it is not needed; but in cases of hæmoglobinuria it is valuable in con-

firming the presence of blood pigment. Filter the urine if it be not clear; dilute it with water if it absorb too much light. With the spectroscope look at a bright light through a test-tube filled with the urine. If recent blood be present, the two absorption bands of oxyhæmoglobin will be noted (Fig. 17), one at the

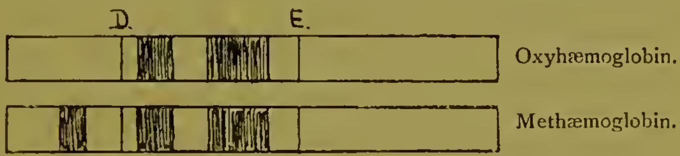

FIG. 17.—Spectra of Blood in Urine.

junction of the yellow with the green, the other in the middle of the green rays of the spectrum. Blood that has been longer mixed with the urine gives the three bands of methæmoglobin (Fig. 17), one in the red in addition to the two of oxyhæmoglobin.

Fallacies :

None, except that the test requires care and skill in carrying it out. Other substances have spectroscopic bands, but none exactly like the above.

Microscope Test.—Examine the centrifugalized deposit for red corpuscles; their presence is the best proof of hæmaturia.

Fallacies :

(1) In alkaline urine the red corpuscles soon swell up and disappear.

(2) In paroxysmal hæmoglobinuria and in blackwater fever blood pigment is present, but no red corpuscles.

Importance of Blood in the Urine.—Except in women at the menstrual period, the presence of blood is always pathological. Laboratory tests cannot by themselves decide what part of the genito-urinary tract the blood comes from; but when the blood is intimately mixed with the urine, the source is usually the kidney, whilst when it comes chiefly at the beginning or the end of micturition, the source is elsewhere. Methæmoglobin in fresh urine indicates renal hæmorrhage rather than vesical. Hæmaturia does not always indicate a lesion in the urinary tract, for it may occur in the blood diseases, such as leuchæmia or purpura.

3. Pus.—The deposit is opaque and white; in small quantities it may be mistaken for mucus; in larger for phosphates or for colourless urates. Urates disappear on warming, pus remains; phosphates increase, but clear up with acetic acid. Mucus and pus can be distinguished by the following tests:

Liquor Potassæ Test.—To 1 inch of the suspected deposit in a test-tube add 1 inch of liquor potassæ; pour the mixture from one test-tube into another. Pus will have partially dissolved and become 'ropy' and gelatinous.

Fallacy:

The test will not detect small quantities of pus.

Ozonic Ether Test.—To 1 inch of the deposit in a test-tube add 1 inch of ozonic ether. On gently shaking, numbers of small bubbles of liberated oxygen will be seen rising through the fluid.

Fallacies :

(1) Blood also causes bubbling with ozonic ether.

(2) Normal urine causes a few bubbles to come off, so that the test will not detect small quantities of pus.

Guaiacum Test.—Performed as for blood. Even without adding ozonic ether, a green-blue colour appears after a few minutes.

Fallacies :

Blood, iodides, saliva (p. 20).

Microscope Test.—Under $\frac{1}{6}$ inch objective pus corpuscles appear as leucocytes which have undergone granular or fatty degeneration. Their nuclei can be brought out by adding a drop of acetic acid (Fig. 13, p. 10.)

Fallacy :

If it be remembered that a few leucocytes may accompany simple inflammatory conditions of the urinary tract, there is no fallacy. Presence of numerous leucocytes in the urine indicates pus, and is the only satisfactory test.

Importance of Pus.—It is always pathological. Small quantities of pus derived from the urethra or vagina may occur in acid urine. Much pus in an acid urine indicates renal suppuration, in which case the acidity is maintained by healthy urine from the sound kidney. If both kidneys be affected, or if there be cystitis, the purulent urine will be alkaline and ammoniacal.

4. SUGAR.—Two sugars may occur in urine, glucose, and lactose. The following tests are for glucose.

Fehling's Test.—Fehling's solution is made by mixing equal parts of the following fluids:

Copper Sulphate Solution.

Crystallized copper sulphate	..	34·64	grammes
Distilled water up to ..	..	500	c.c.

Rochelle Salt Solution.

Potassium hydrate ..	..	125	grammes
Sodium potassium tartrate (Rochelle salt)	..	173	,,
Distilled water up to ..	..	500	c.c.

The copper sulphate solution is pale green, that of Rochelle salt colourless. On mixing the two the potassium hydrate converts the copper sulphate into cupric hydrate, and the latter is kept in solution by the Rochelle salt, giving a deep blue transparent fluid. The separate fluids keep good any length of time; mixed, they slowly decompose. They should therefore be kept in separate bottles and mixed as required. To perform the test, boil 1 inch of Fehling's solution in a test-tube; if good, it remains clear. In another test-tube boil 1 inch of urine; without further boiling pour the hot urine slowly into the hot Fehling's solution. If glucose be present, it will reduce the cupric hydrate to cuprous oxide, which comes down as an orange precipitate.

Fallacies:

(1) Lactose reduces Fehling's solution, but may be distinguished by the fermentation and the phenylhydrazine tests.

(2) Slight reduction of Fehling's solution, causing first a greenish colour of the solution, and

then a dark greenish-yellow precipitate, may be caused by other reducing substances in the urine, namely :

Uric acid.	Creatinin.
Hippuric acid.	Glycuronic acid.
Xanthin.	Glycosuric acid.
Hypoxanthin.	Alkapton.

And the products of certain drugs :

Chloral.	Carbolic acid.
Chloroform.	Salicylates.
Glycerine.	

None of these ferment. Error from these sources is not likely to occur if the two fluids be boiled separately and then mixed. Glucose and lactose alone will give the reduction without further boiling.

(3) Albumin interferes with Fehling's test. If it be present, add acetic acid, boil, filter off the coagulated albumin, and test the filtrate.

(4) In ammoniacal urines the free ammonia may prevent precipitation of cuprous oxide. Before testing with Fehling's solution, boil well to expel the ammonia.

Trommer's Test.—Trommer's solution is a mixture of copper sulphate and potassium hydrate. It differs from Fehling's in not containing Rochelle salt. The test is performed in precisely the same way, and has the same fallacies.

Böttger's Test.—The reagent used is the following :

Caustic soda	10 grammes
Sodium potassium tartrate (Rochelle salt)	4 ,,
Bismuth subnitrate	2 ,,
Distilled water	100 c.c.

To 2 inches of urine in a test-tube add 5 drops of the reagent; boil for five minutes. If glucose be present the bismuth is reduced, and a fine black precipitate of bismuth suboxide appears.

Fallacies:

Lactose and glycuronic acid also give the test; but uric acid, creatinin, and xanthin bases which give some reduction with Fehling's test give none with Böttger's.

Picric Acid Test.—To 1 inch of urine in a test-tube add $\frac{1}{4}$ inch of saturated solution of picric acid and 5 drops of caustic potash. Boil. If glucose be present the solution darkens in colour, becoming first dark brown and then almost black, from the reduction of picric to picramic acid.

Fallacies:

(1) Lactose gives the test also.

(2) In normal urine creatinin causes some darkening, so that as a sugar test picric acid is not very delicate.

(3) Impure picric acid darkens when boiled with caustic potash. The solution should therefore be tested before it is added to the urine.

Moore's Test.—Mix 1 inch of urine with 1 inch of liquor potassæ in a test-tube and boil. If sugar be present a ruddy brown colour develops, going on to a dark brown and almost black.

Fallacies:

(1) The test will not detect small quantities of sugar.

(2) Carboluria, alkapton, and indican also cause darkening with potash.

Phenylhydrazine Test.—To 2 inches of urine in a test-tube add as much phenylhydrazine chloride as will lie easily on a threepenny-piece, and a rather larger quantity of sodium acetate. Stand the test-tube in boiling water for twenty minutes, then allow it

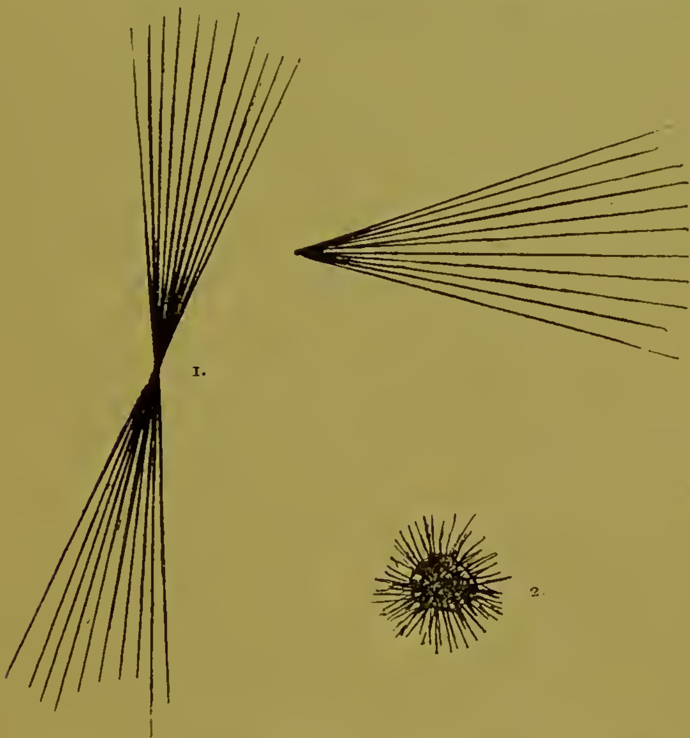

FIG. 18.—(1) Phenylglucosazone crystals; (2) Phenyl-lactosazone crystals.

to cool. If glucose be present, phenylglucosazone crystals appear, in the form of fans and sheaves of long bright yellow needles seen by the low power (Fig. 18).

Fallacies:

(1) Lactose gives yellow needles of phenyl-lactosazone, but they are smaller, and arranged

less as fans and sheaves than as spheres with radiating spines (Fig. 18).

(2) It is said that glycuronic acid does not give the phenylhydrazine test. It does so sometimes, however, the crystals being similar to those of glucose, but more difficult to obtain.

Fermentation Test.—Triturate a piece of good yeast, the size of a small marble, with 250 c.c. of urine. Fill a Southall's ureometer (Fig. 24, p. 45) with the mixture, allow it to stand in a warm place for twenty-four hours. Glucose, if present, will ferment, and the CO_2 given off will collect as a bubble at the top of the closed limb of the ureometer.

Fallacies:

None. No other reducing body in the urine will ferment with yeast. Lactose does not.

SUMMARY OF SUGAR TESTS.

For Glucose.	*For Lactose.*
Fehling's test serves all ordinary purposes.	Gives Fehling's test.
Phenylhydrazine test is a good confirmation.	Gives crystals with the phenylhydrazine test somewhat different to those of glucose.
Fermentation test, the only absolute proof of the presence of glucose.	Will not ferment with yeast.

Moore's, Trommer's, Böttger's, and the picric acid tests are confirmatory.

Importance of Sugar in the Urine.—It is important to know if the sugar be glucose. Lactose is not pathological, but is closely related to pregnancy in women, occurring both before and after parturition. Glucose in appreciable quantities is pathological. Its presence may be temporary, as after

alcoholic excess, an injury to the head, or a vascular lesion of the brain. It may be intermittent in the gouty or alimentary glycosuria of elderly people, or it may indicate true diabetes mellitus, in which case it is usually associated with acetone and diacetic acid.

5. ACETONE.

Sodium Nitroprusside Test.—To 1 inch of urine in a test-tube add 1 inch of caustic soda and ½ inch of 1 per cent. solution of sodium nitroprusside. A ruby red colour appears. Add acetic acid. If acetone be present the colour deepens to a rich port-wine colour.

Fallacies :

(1) If potash or ammonia be used instead of soda, the test may fail.

(2) Creatinin gives a similar ruby red with the nitroprusside and caustic soda, but on adding acetic acid the colour, instead of deepening, disappears.

Iodoform Test.—To 1 inch of urine in a test-tube add 5 drops of caustic potash ; warm to body heat ; add iodine solution until the liquid is yellowish brown ; warm gently. If acetone be present iodoform will form, and may be recognised by its odour or by its yellow crystals.

Fallacy :

The iodoform test is less delicate than the sodium nitroprusside.

Importance.—In the absence of sugar, acetone has been found in lobar pneumonia, tetany, gastric ulcer, and other conditions, and in these seems to have no importance ; associated with glycosuria,

acetone indicates true diabetes mellitus with liability to coma.

6. Diacetic Acid.—To 1 inch of urine in a test-tube add liquor ferri perchlor. (B.P.) drop by drop. A white precipitate of iron phosphate forms. Later, if aceto-acetic acid be present, the liquid becomes claret coloured, but is decolourized by warming.

Fallacies:

If carbolic acid, salol, salicin, salicylates, antipyrin, or other drug containing a phenyl basis be taken, the urine gives a similar reaction with ferric chloride. The colour due to these causes does not disappear on warming.

Importance.—Similar to that of acetone. In diabetes diacetic acid is seldom present without acetone, though acetone may occur without diacetic acid.

7. β-oxybutyric Acid.—Ferment the urine with yeast to remove glucose. Filter. Evaporate on a water-bath to a syrupy consistence. Add an equal bulk of strong sulphuric acid, distil the mixture, and collect the distillate in a test-tube. If β-oxybutyric acid be present, crystals of α-crotonic acid will form, and may be recognised by determining their melting-point, 72° C.

Importance.—There is little need to test for this acid, because it is easier to test for the acetone and diacetic acid which are associated with it. It may occur in certain febrile states. In glycosuria it has the same significance as has acetone, namely, true diabetes mellitus with liability to coma.

8. Glycuronic Acid.—There is no good clinical test for this acid; it may be mistaken for sugar because it reduces Fehling's and Böttger's solutions, and can form crystals with phenylhydrazine not unlike those of phenylglucosazone. But *it will not ferment.*

Importance.—It occurs in normal urine to a small extent, and is increased after giving chloral, butyl chloral, nitrobenzol, camphor, and after chloroform narcosis.

9. Bile Pigments.—The colour varies from brownish yellow to dark green, and may be simulated by carboluria, alkaptonuria, hæmaturia, indicanuria, melanuria; but if the urine be shaken up the colour of the froth also is greenish when bile pigments are present; not so in the other cases.

Gmelin's Test.—Upon a white porcelain slab put 1 drop of the urine, and close to it a drop of nitric acid. At their point of coalescence a play of colours, yellow, red, green, blue, yellow, will occur if bile pigments be present. The test may also be performed in a similar way on filter-paper. Or if 1 inch of nitric acid be put into a test-tube, and the urine carefully poured on to its surface, the play of colours is readily seen at the junction of the two fluids.

Fallacy:

None.

Iodine Test.—Take 1 inch of urine in a test-tube; gently pour 10 per cent. solution of iodine in alcohol on to its surface. In the presence of bile pigments a bright green ring appears between the two liquids.

Fallacy:

The test is not so delicate as Gmelin's.

Importance.—They indicate jaundice, but not its cause.

10. BILE SALTS.

Pettenkofer's Test.—To 1 inch of urine in a test-tube add 1 c.c. of strong solution of cane-sugar and strong sulphuric acid drop by drop, holding the end of the test-tube in running water to keep it cool. In the presence of bile salts a rich purple colour appears.

Fallacies :

(1) Normal urine, with strong sulphuric acid, gives a brownish-purple colour, which may be mistaken for a positive reaction. A control test should always be made with normal urine.

(2) It is common to find bile pigments by Gmelin's test, and yet not to find bile salts by Pettenkofer's.

Sulphur Test.—Fill a test-tube to the top with urine; sprinkle finely-powdered commercial sulphur on to its surface. Whereas in healthy cold urine the sulphur floats, even minute traces of bile salts so alter the surface tension that the sulphur sinks.

Fallacies :

(1) If the urine be warm, the sulphur sinks even in the absence of bile salts.

(2) The test is almost too delicate. By it the majority of urines are shown to contain traces of bile salts, so that the very delicacy of the test detracts from its clinical value.

Importance.—Bile salts are not known to give evidence which is not equally afforded by the bile pigments.

11. CHLORIDES.—To 1 inch of urine in a test-tube add

5 drops of nitric acid and 2 drops of silver nitrate solution. Chlorides give a dense white precipitate. The nitric acid is to keep the phosphates and sulphates in solution.

Fallacy:

Albumin is precipitated by the nitric acid.

Importance.—Some deficiency of chlorides occurs in many fevers; but in lobar pneumonia they may be almost or quite absent, assisting in diagnosis from empyema or simple pleurisy. Presence of chlorides does not exclude lobar pneumonia, and they may be absent in other conditions; for example, suppurative peritonitis, or in absolute starvation.

12. INDICAN.—To 2 inches of urine in a test-tube add 1 inch of strong hydrochloric acid and 5 drops of fuming nitric acid; boil; allow to cool; add $\frac{1}{2}$ inch of chloroform, and shake up thoroughly. If indican be present, the chloroform when it has again sunk to the bottom of the test-tube will be tinged either blue or red.

Fallacies:

(1) Albumin interferes with the test. It should, if present, be first removed by acidifying the urine with acetic acid, boiling, and filtering off the coagulated proteid.

(2) Iodides, if present, will colour the chloroform blue.

Importance.—'Indican' is a mixture of potassium indoxyl and skatoxyl sulphates, derived from the indol and skatol which arise from intestinal decomposition. Indicanuria thus points to putre-

faction in the intestine from chronic constipation, from acute or ulcerative colitis or enteritis, and other similar conditions.

13. UROBILIN.—Examined with the spectroscope, an absorption band between the green and blue is typical of urobilin (Fig. 19). To 1 inch of urine in a test-tube add ½ inch of strong ammonia, and then zinc

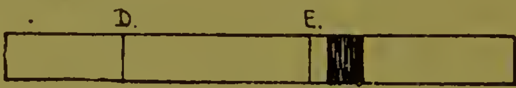

FIG. 19 —Urobilin Spectrum.

chloride solution drop by drop; filter. If urobilin be present the filtrate will be fluorescent, and will show the spectroscopic band more markedly than before.

Importance.—In healthy urine the chief pigment is urochrome, not urobilin. The latter increases in disease, but is not typical of any special illness. It may occur in almost any fever, in some affections of the liver, tuberculous peritonitis, pernicious anæmia, and in many other conditions.

14. THE DIAZO REACTION.—The following two solutions are needed, and each should be fresh :

Sulphanilic Acid Solution.

Hydrochloric acid	5 c.c.
Distilled water	100 ,,
Sulphanilic acid to saturation.	

Sodium Nitrite Solution.

Sodium nitrite	0·5 gramme
Distilled water	100 c.c.

To 1 inch of urine in a test-tube add 1 inch of

sulphanilic acid solution and 5 drops of the sodium nitrite. Add ammonia till alkaline, and shake thoroughly. The test is positive if the liquid becomes port-wine colour *and the froth rose red.* The rose colour of the froth is essential.

Importance.—The reaction is common in the third week of typhoid fever, but is not always present. It also occurs in tuberculosis. A positive diazo reaction in phthisis is a bad sign; but if the reaction has at one time been found, and later has disappeared, it indicates improvement in the patient.

15. Albumose.—First remove albumin by acidifying the urine with acetic acid, boiling and filtering. On to the surface of 1 inch of nitric acid in a test-tube gently pour some of the cooled filtrate. If albumose be present, a white ring will appear at the junction of the fluids; the ring will disappear on warming, to reappear again on cooling.

Fallacies :

Urea nitrate; uric acid; copaiba; mucin; as in the case of albumin (p. 17).

Importance.—Albumosuria occurs to a slight degree in many fevers, notably pneumonia, where it has no known significance. It also occurs when there are collections of pus under pressure, as in pelvic or appendicular abscess, or empyema. In large quantities it appears to be pathognomonic of endosteal sarcoma of bone.

16. Mucin.—Add to the suspected deposit a few drops of acetic acid; the cloud becomes more marked, and still more so on boiling. It is distinguished from

phosphates by not clearing up with acetic acid; from pus by the absence of leucocytes under the microscope; from colourless urates by not clearing up on warming.

> *Importance.*—Mucin, or an allied body nucleo-albumin, is the cause of the flocculent cloud which appears in many healthy acid urines on standing. If its amount be large it indicates catarrh of the urinary passages, particularly of the bladder.

17. CARBOLURIA, ALKAPTONURIA, AND MELANURIA.—These three conditions account for various greenish-brown, greenish-black, brownish-black, and blackish tints that are sometimes observed in urines. They may be mistaken for bile or blood pigments. The urine is frequently normal in colour when passed, but darkens when exposed to the air. In carboluria this is due to oxidation of hydrochinone and pyrocatechin; in alkaptonuria to uroleucic and homogentisic acids; in melanuria to melanogen, which becomes melanin on standing. There are no easy clinical tests for determining which pigments are present, but the following reactions will indicate the probable nature of a given case.

In Carboluria and Alkaptonuria: Ferric chloride gives a white precipitate of phosphates and a violet-coloured solution. Bromine water gives a dense pale yellow precipitate of tribromophenol. After boiling with hydrochloric acid, and then testing with Fehling's solution, there is partial reduction. Böttger's reagent is not reduced. Gently warmed with caustic potash, the dark colour deepens. Rapidly heated in an open

test-tube, the hydroquinone forms violet fumes, which condense as an indigo-blue sublimate.

In Melanuria: Ferric chloride: the precipitate of phosphates carries down the melanin as a dark gray deposit, soluble in excess of ferric chloride. Bromine water gives a dense yellow precipitate, which slowly blackens. There is no reduction with Fehling's solution. Gently warmed with caustic potash, the dark colour deepens. Caustic potash, sodium nitro-prusside, and acetic acid give a deep blue colour.

Importance.— Carboluria may follow carbolic acid poisoning; some patients are so susceptible that carbolic acid lotion or dressings, carbolic acid or salicylates by the mouth, may cause the condition.

Alkaptonuria has occurred spontaneously in healthy persons; and it is an occasional accompaniment of tuberculous peritonitis, malignant cachexias, and affections of the liver.

Melanuria may occur in patients suffering from melanotic sarcoma; but it is not constant in such cases, and it is sometimes marked in other wasting diseases.

18. Lead.—Add 1 gramme of ammonium oxalate to 150 c.c. of urine; immerse a strip of bright magnesium wire in it for twenty-four hours. Wash the strip in distilled water; dissolve it in dilute nitric acid; test the solution for lead as follows:

(1) A drop of HCl added gives a dense white precipitate of $PbCl_2$.

(2) Chromate of potash gives a bright yellow precipitate of lead chromate.

(3) Ammonium sulphide in excess gives a black precipitate of lead sulphide.

(4) Potassium iodide gives a bright yellow precipitate of lead iodide.

Importance.—Lead is excreted in the urine, and may be found there in cases where plumbism is suspected, even in the absence of a blue line upon the gums.

19. SOME DRUG REACTIONS IN THE URINE :

Antipyrine.—Urine may be red, suggesting blood. No guaiacum reaction. With ferric chloride purple, not disappearing on heating. With Fehling's solution, partial reduction on continued boiling.

Carbolic Acid.—Urine darkens on standing; may suggest blood or bile; no guaiacum reaction; no Gmelin's test. With ferric chloride, purple, not disappearing on heating. With bromine water, dense yellow precipitate of tribromophenol. With Fehling's solution, partial reduction on continued boiling.

Chloral and Chloroform cause increase of glycuronic acid. With Fehling's solution, reduction; no fermentation with yeast.

Copaiba.—With nitric acid, a white ring which might be mistaken for albumin. Ring redissolved by alcohol. Add 2 drops of HCl to urine in a test-tube; copaiba gives a pink colour.

Iodides.—With guaiacum, blue colour; may suggest blood. Add HCl and shake up with chloroform; the latter takes up the liberated iodine, becoming violet; indican gives a similar reaction, but requires HNO_3 as well as HCl.

Rhubarb.—Reddish-brown urine may suggest blood. No guaiacum reaction. With caustic potash, red colour deepens; on now adding HCl, it becomes yellow.

Salicylates and Salol.—With Fehling's solution, partial reduction on continued boiling. With ferric chloride a purple colour, not disappearing on warming, and thus unlike diacetic acid. They may cause alkaptonuria, the urine darkening on standing.

Santonin.—Bright yellow urine; caustic potash turns it bright pink.

Sulphonal. — Sometimes causes hæmatoporphyrinuria, the urine being port-wine coloured, suggesting blood, and yet not giving the guaiacum test. Add 2 drops of acetic acid to some urine, and shake it up with an equal volume of ether. This extracts the pigment, which, examined spectroscopically, gives the four-banded spectrum of alkaline hæmatoporphyrin (Fig. 20). When HCl is added, it changes to that of

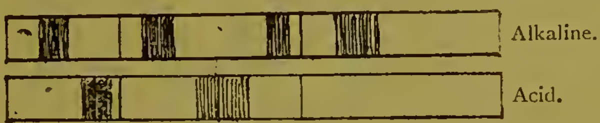

FIG. 20.—Hæmatoporphyrin Spectra.

acid hæmatoporphyrin, namely, a band in the orange and another between the yellow and green (Fig. 20).

IV. METHODS OF ESTIMATING VARIOUS CONSTITUENTS OF URINE.

1. Estimation of Albumin.

Esbach's Method. — An Esbach's albuminometer (Fig. 21) and Esbach's fluid are needed. The former is a special test-tube, graduated in its lower part by marks which correspond to parts per thousand, and with two other marks, U and R respectively.

Esbach's fluid is as follows:

Picric acid	10 grammes
Citric acid	20 ,,
Distilled water to	1,000 c.c.

Fill the tube with urine to the mark U, and add reagent to the mark R; cork, and gently invert several times. Stand vertically for twenty-four hours. The mark then corresponding to the upper level of the precipitate gives the parts per thousand of albumin.

Fig. 21.—Esbach's Albuminometer.

Fallacies:

Occasionally the albumin floats instead of sinking. In that case shake the tube up again and leave for another twenty-four hours.

The test is one of sedimentation, so that if left for more or less than twenty-four hours the reading will be proportionately less or more than it should be.

The higher graduations become closer and closer together, so that the difference for each part per 1,000 over 5 is difficult to measure accurately. If more than 5 parts

per 1,000 are present, dilute the urine once, twice, thrice, as may be necessary, and multiply the amount then found by 1, 2, or 3 respectively.

The picric acid also precipitates pigments and aromatic compounds. The amount of these is negligible in proportion to large amounts of albumin, but with a reading less than 1 part per 1,000 the error due to pigments is great. The method gives accurate readings for albumin only between 1 per cent. and 5 per cent., and only when allowed to stand exactly twenty-four hours.

Roberts' Method.—This depends on the fact that it requires three minutes for the white ring with nitric acid to appear when there is 1 part of albumin in 30,000; no special apparatus is required. Dilute the urine ten, twenty, thirty times, and so on, and after each dilution test with nitric acid. Suppose the white ring required three minutes to appear after thirty times dilution, there would be 30 parts of albumin per 30,000, or 1 part per 1,000.

2. ESTIMATION OF SUGAR.

Pavy's Method.

Pavy's Solution.

Copper sulphate	..	..	..	4·16 grammes
Rochelle salt	..	..	..	20·4 ,,
Caustic potash	..	..	..	20·4 ,,
Strong ammonia	..	..	..	300 c.c.
Water to ..	..	..	..	1,000 ,,

10 c.c. are reduced by 0·005 gramme of glucose.

Pavy's solution differs from Fehling's in containing ammonia, which prevents the precipitation of cuprous oxide. The fresh solution is blue; when reduced, colourless. Fill a burette with the urine diluted 1 in

20. Put 10 c.c. Pavy's solution and 20 c.c. water into a 200 c.c. flask fitted with a cork bored with two holes. Through one hole pass the nozzle of the burette, through the other a glass tube 4 inches long, which reaches just into the flask at one end, and is open to the air at the other (Fig. 22). Heat the flask until the Pavy solution just boils; keep it simmering; turn the stopcock of the burette so that the diluted urine drops in steadily at the rate of 4 drops per second. Do not alter this rate of dropping, but watch the colour of the fluid in the flask; it will presently fade. When the Pavy solution is just colourless, turn the stopcock off, and read off the amount of urine added. The calculation is as follows: The 10 c.c. Pavy solution require 0·005 gramme glucose for complete reduction. Suppose 3 c.c. of the twenty times diluted urine were added;

FIG. 22.—Pavy's Estimation of Sugar.

∴ in 3 c.c. of twenty times diluted urine there are 0·005 gramme of glucose.

∴ in 1,000 c.c. of twenty times diluted urine there are $\frac{0{\cdot}005 \times 1{,}000}{3}$ grammes of glucose.

∴ in 1,000 c.c. of original urine there are $\frac{0{\cdot}005 \times 1{,}000 \times 20}{3}$ grammes of glucose.

$= 33{\cdot}3$ per cent.

The method is liable to considerable personal error; it gives strictly comparable results, however, if always performed by the same person and in the same way.

If the flask be not corked as described, the ammonia rapidly boils off, and a thick yellow deposit of cuprous oxide comes down.

The urine should always be diluted to such an extent that not less than 1 c.c. and not more than 3 c.c. are required to decolourize the 10 c.c. of Pavy's solution.

(*N.B.*—After the estimation, the blue colour will return to the reduced Pavy's solution, owing to reoxidation of the cuprous oxide in the flask.)

Gans' Method.—This is less accurate than Pavy's, but may be employed in general practice as it is less complicated. It depends on the volume of CO_2 evolved on fermentation with yeast. To 10 c.c. urine add a small quantity of finely-triturated yeast; dilute to 100 c.c. with water; mix thoroughly. Transfer 10 c.c. to the bulb of a Gans' apparatus (Fig. 23), and insert the stopper. The graduated vertical limb is open at the top, and there is an arrangement, easily understood when the apparatus is seen, for adjusting the level of the fluid to the mark 0. Stand in a warm place till next day. The CO_2 evolved in the bulb will have forced the fluid up in the vertical limb, and the graduation corresponding to its upper level gives the parts per cent. of sugar in the original urine. Some of the CO_2 escapes, of course; but this

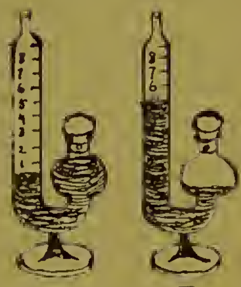

FIG. 23.—Gans' Apparatus.
A, before fermentation
B, after.

has been allowed for, the graduations having been made experimentally with known percentages of glucose.

The method avoids error from presence of other reducing bodies than sugar in the urine.

The chief inaccuracy is the variation in volume of gas in the bulb with changes in temperature and barometric pressure. The estimation must therefore always be made at the same temperature as far as possible.

Specific Gravity Method.—This is easy, and affords approximate results. It is very useful in private practice. Mix some finely triturated yeast with a quantity of urine. Take the specific gravity of the mixture. Stand in a warm place for twenty-four hours, and take the specific gravity again. Glucose will have fermented, and the specific gravity will have fallen. Every degree of specific gravity lost corresponds to 1 grain per ounce of glucose. For example:

Specific gravity—before fermentation	1040
,, ,, after ,, ...	1022
Grains per ounce of glucose ..	18

3. Estimation of Urea.

Sodium Hypobromite Method with Southall's Ureometer.

Hypobromite Solution.

Caustic soda	100 grammes
Water	250 c.c.
When cool, add bromine	25 ,,

Sodium hypobromite (NaBrO) is formed, and caustic soda is in excess.

Fill the bulb of the ureometer (Fig. 24, A) with this solution, and tilt the apparatus so as to displace all the air in the closed vertical limb. Measure off 1 c.c. of urine with the pipette (Fig. 24, B). A good way of doing this is to expel *all* the air from the elastic nipple, plunge the end of the glass pipette into the urine, and release the nipple so that urine is drawn up beyond the mark. Raise the pipette out of the urine, and by gentle pressure on the nipple expel the excess down to the mark 1; then plunge the tip into distilled water and release the nipple again. A layer of water (*a*, *b*) will be drawn up between the urine (*b*, *c*) and the orifice of the pipette, and at the same time the outside of the latter will have been washed clean. Now insert the end of the pipette through the bulb of the ureometer till it reaches the bottom of the vertical limb; slowly compress the nipple, expelling first the water (*a*, *b*) and then the urine (*b*, *c*) into the hypobromite solution. As soon as the urine mixes with the hypobromite, bubbles of gas come off, the urea being decomposed into CO_2, H_2O, and N_2. The CO_2 is dissolved by the excess of soda; the H_2O condenses; only the N_2 rises to the top of the vertical limb.

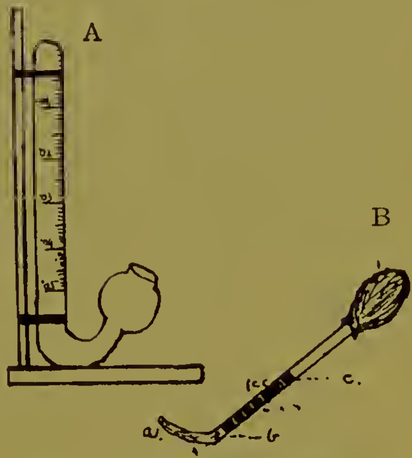

FIG. 24.—Southall's Ureometer and Pipette.

Allow the froth to settle down; read off the graduation corresponding to the lower level of the gas. There are two sets of graduations, one showing parts per cent., the other grains per ounce.

Importance.—A healthy man excretes about 400 grains, or 30 grammes, of urea in twenty-four hours; but there are wide limits according to the food taken. In the majority of cases a small percentage of urea means one of two things: either little nitrogenous food is being taken, or the urine is very dilute. This is apparently as true in Bright's disease as in other illnesses. In certain fevers more urea is excreted than corresponds to the nitrogen in the food, owing to breaking-down of the patient's own tissues. This may also be the case in diabetes, though no deductions can be drawn unless the nitrogen in the food is also known.

The hypobromite method is only approximate; more nitrogen is given off than corresponds to the urea alone, less than to the total nitrogen in the urine. For exact estimations of the urea, the complicated Mörner-Sjöquist process must be used (see Schäfer's 'Text-Book of Physiology,' vol. i.).

4. ESTIMATION OF URIC ACID.

Hopkins' Potassium Permanganate Method.—To 100 c.c. of urine in a flask add 35 grammes of ammonium chloride. Allow to stand for fifteen minutes after this has dissolved. Insoluble ammonium urate is precipitated. Filter. Wash the precipitate with hot ammonium chloride solution. Open out the filter-paper, and with hot distilled water wash the pre-

cipitate into a graduated 100 c.c. flask. Dilute accurately to 100 c.c. with water. Pour into a beaker, add 20 c.c. strong sulphuric acid, and from a burette drop in standard $\frac{N}{20}$ permanganate solution made up as follows:

Potassium permanganate ..	..	7·9 grammes
Distilled water ..	..	.. 1,000 c.c.

1 c.c. corresponds to 0·00375 gramme uric acid.

The crimson fluid as it drops in is at first rapidly decolourized; presently the colour disappears more slowly. When the liquid in the beaker retains a distinctly pink colour for fifteen seconds the end reaction is reached. Suppose 8 c.c. of permanganate have been added;

∴ in 100 c.c. of urine there are $8 \times 0{\cdot}00375$ gramme uric acid $= 0{\cdot}03$ per cent. uric acid.

The average total uric acid in twenty-four hours is 0·5 gramme.

(*N.B.*—For very accurate estimations, certain 'corrections' have to be made.)

5. Estimation of Chlorides.

Volhard's Method.—This consists in adding excess of silver nitrate to precipitate all the chlorides, and determining the excess of silver nitrate with standard solution of potassium sulphocyanide.

Standard Silver Nitrate Solution.

Fused silver nitrate ..	..	29·07 grammes
Distilled water to ..	..	1,000 c.c.

1 c.c. corresponds to 0·01 gramme sodium chloride.

Standard Potassium Sulphocyanide (KCNS) Solution.

Potassium sulphocyanide ..	..	8·29 grammes
Distilled water to ..	..	1,000 c.c.

2 c.c. corresponds to 1 c.c. of standard silver nitrate solution.

A strong solution of iron alum is also required as indicator, and some pure nitric acid.

To 10 c.c. of urine in a standard 100 c.c. flask add 5 c.c. of pure nitric acid and excess of standard silver solution, say 25 c.c., measured from a burette. Dilute with water to 100 c.c. Filter off the precipitated silver chloride. Pipette 50 c.c. of the filtrate into a beaker, add 5 c.c. of iron alum solution; and then standard potassium sulphocyanide solution from a burette. The end reaction is the appearance of a permanent red colour, due to formation of sulphocyanide of iron when all the silver has been precipitated.

Suppose 8 c.c. of potassium sulphocyanide solution were thus needed,

2 c.c. of potassium sulphocyanide solution corresponds to 1 c.c. of standard silver solution;

∴ 8 c.c. of potassium sulphocyanide solution corresponds to 4 c.c. of standard silver solution.

∴ in 50 c.c. of the filtrate there were 4 c.c. of silver solution in excess;

∴ in 100 c.c. there were 8 c.c. of silver solution in excess.

But in the 100 c.c. there were 10 c.c. of urine; therefore of the 25 c.c. of silver solution added to the 10 c.c. of urine, 8 c.c. were in excess.

∴ to precipitate the chlorides in 10 c.c. of urine 25 - 8 c.c. silver solution are required = 17 c.c. But 1 c.c. silver solution corresponds to 0·01 gramme sodium chloride.

∴ 10 c.c. of urine contain 17 × 0·01 grammes of sodium chloride;

∴ 100 c.c. of urine contain $17 \times 0{\cdot}01 \times 10$ grammes of sodium chloride = 1·7 per cent.

The method is exceedingly accurate, and the end reaction very definite.

Upon full diet about 15 grammes of sodium chloride are excreted per diem.

Mohr's Method.—To 10 c.c. of urine in a beaker add 100 c.c. of distilled water and 20 drops of saturated potassium chromate solution. Add standard silver nitrate from a burette; a distinct red colour appears when all the chlorides have been precipitated. Suppose 12 c.c. of silver nitrate solution have been added;

∴ in 10 c.c. urine there are $12 \times 0{\cdot}01$ grammes sodium chloride;

∴ in 100 c.c. urine there are $12 \times 0{\cdot}01 \times 10$ grammes sodium chloride = 1·2 per cent.

6. Estimation of Phosphates.

The following reagents are needed:

Standard Uranium Nitrate Solution.

Uranium nitrate	35·5 grammes
Distilled water to	1,000 c.c.

1 c.c. corresponds to 0·005 gramme phosphoric acid.

Sodium Acetate Solution.

Sodium acetate	10 grammes
Glacial acetic acid	10 c.c.
Distilled water to	100 ,,

Potassium Ferrocyanide (Saturated Solution). — To 50 c.c. of urine add 5 c.c. of sodium acetate solution. Warm to 80° C. Add standard uranium nitrate from a burette. The end reaction is the appearance of a

distinct brown colour when a drop of the fluid in the beaker is added to a drop of potassium ferrocyanide upon a white slab.

Suppose 11 c.c. of uranium nitrate have been added; then

In 50 c.c. of urine there are $11 \times 0{\cdot}005$ grammes of phosphoric acid;

$\therefore$ in 100 c.c. of urine there are $11 \times 0{\cdot}005 \times 2$ grammes of phosphoric acid $= 0{\cdot}11$ per cent.

On full diet the average is $3{\cdot}5$ grammes per diem.

CHAPTER II.

EXAMINATION OF THE BLOOD.

THIS consists of the following parts :

1. Enumeration of the red corpuscles.
2. Enumeration of the white corpuscles.
3. Estimation of the hæmoglobin.
4. Examination of blood-films for :
   (*a*) Changes in the red corpuscles.
   (*b*) Differential count of the white corpuscles.
   (*c*) Presence of parasites.
5. Estimation of the specific gravity.
6. Examination for : (1) Lipæmia. (2) The Widal reaction of typhoid fever.

Blood is best obtained from the lobule of the patient's ear, which, after cleaning with soap and water and thorough drying, should be held firmly at its base between the finger and thumb of the physician's left hand, and given one *rapid* deep puncture with the needle. An ordinary straight surgical needle answers admirably, but special needles are sold for the purpose, and a very convenient form for nervous patients is enclosed in an adjustable sheath (Fig. 29 G, p. 59). The blood should well up freely, and squeezing should be avoided lest lymph be expressed with it.

Enumeration of the Red and White Corpuscles.

The *Thoma-Zeiss Hæmocytometer* consists of: (1) Counting chamber; (2) specially ground cover-glass; (3) red corpuscle pipette; (4) white corpuscle pipette.

The counting chamber (Fig. 25) is made of an outer flat ring of glass, separated by a circular trench from a central platform, both firmly cemented to a glass slide. The outer ring is higher than the platform, so that the cover-glass, resting flat upon the former, is separated from the latter by $\frac{1}{10}$ millimetre. The centre of the platform is ruled into minute squares, which are grouped into sets of sixteen by double rulings (Fig. 26). Each such 'set' is a 'big' square, of which there are usually sixteen. *Zappert* has introduced a modification with double this number. The side of a small square is $\frac{1}{20}$ millimetre; the area $\frac{1}{20} \times \frac{1}{20}$ square millimetre; and the cubic space over each small square between the platform below and the cover-glass above is $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cubic millimetre.

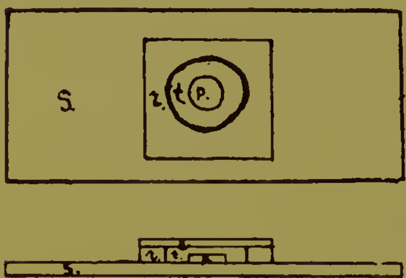

Fig. 25.—Blood-counting Chamber.

t = trench, *p* = platform, *r* = ring, *s* = slide, *c* = cover-glass.

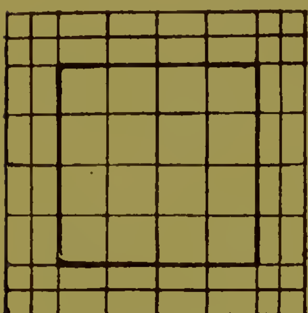

Fig. 26.—One of the 'big' Squares.

The stem of the red corpuscle pipette (Fig. 27) is graduated into tenths up to a mark 1; the bulb con-

tains a glass bead to facilitate mixing; above the bulb is a mark 101.

The white corpuscle pipette is similar, but the bore is wider, the bulb smaller, and the upper graduation is 11 instead of 101.

The diluting fluids required are the following:

Hayem's Fluid.—For dilution in counting red corpuscles:

Mercury perchloride	..	0·25	gramme
Sodium chloride	..	0·5	,,
Sodium sulphate	..	5·0	grammes
Distilled water ..	..	100	c.c.

Or one may use a 0·8 per cent. solution of common salt, which is isotonic with human blood, and therefore neither lakes nor crenates the corpuscles.

Thoma's Fluid.—For dilution in counting white corpuscles:

Acetic acid (B.P.)	..	..	1	c.c.
Gentian violet solution		..	0·5	,,
Distilled water	..	..	100	,,

FIG. 27.—Hæmocytometer Pipette.

In this fluid the red corpuscles are laked; their ghosts alone are seen. The white corpuscles stand boldly out, their nuclei being just tinged with the stain.

It is convenient to reserve for blood-counts, either upon a small tray or in a case, five wide-necked stoppered bottles. In the first keep Hayem's fluid, in the second Thoma's fluid, in the third, fourth, and fifth distilled water, absolute alcohol, and ether respectively. The three latter are for *cleaning the pipettes.*

Never postpone this, lest the blood dry and clog the capillary bore. Immediately a pipette is done with, blow out the contents; draw up distilled water from bottle 3 into the bulb, and blow it out again; repeat this three times. Now draw up absolute alcohol from bottle 4 in a similar way three times. Finally, draw up ether from bottle 5 three times; after blowing out the ether the last time, draw air through the bulb. The water cleans the pipette, the alcohol dehydrates it, the ether replaces the alcohol, itself rapidly evaporates, and leaves the apparatus clean and dry ready for use. Done at once, the cleaning takes a minute; left till next day, perfect cleaning may be impossible.

To count the Red Corpuscles.—Prick the ear, wipe away the first drop of blood, wait till a second the size of a hemp seed has collected, immerse the end of the pipette in it, and draw blood up to the mark 0·5. Quickly wipe the end of the pipette, plunge it into bottle 1, draw up Hayem's fluid to the mark 101; pinch the rubber tube, hold the pipette horizontally, and rapidly roll the bulb to and fro to mix the contents thoroughly. The blood dilution is 1 in 200. The upper mark is 101, and not 100, because the Hayem's fluid in the stem up to the point 1 takes no part in the mixture. Get rid of it in the first drop that is blown from the pipette, and then put part of the next drop on to the central platform of the counting chamber. With a little practice it is easy to judge the exact amount required for this. Apply the cover-glass, and press it down. If the fluid runs off the platform into the trench, too big a drop has been taken. Part of a drop only is required; when covered,

it should just extend to the margin of the platform, but not over the edge. If it do so, or if a bubble of air get in, clean the counting chamber, and take a fresh sample. Enumerate the corpuscles under a $\frac{1}{6}$ inch objective, counting the number in each of the sixteen small squares which make up one big square; and, for accuracy, count at least eight sets of sixteen small squares. The variations are considerable. The following is an example of a count in one big square:

3	8	10	5	Total 100.
7	7	9	1	
6	1	13	5	
10	6	5	4	

Corpuscles often overlap dividing lines. Such are apt to be counted twice, unless a rule be made to count into a square those which overlap its top and right-hand, but not those over the bottom and left-hand, sides (Fig. 28).

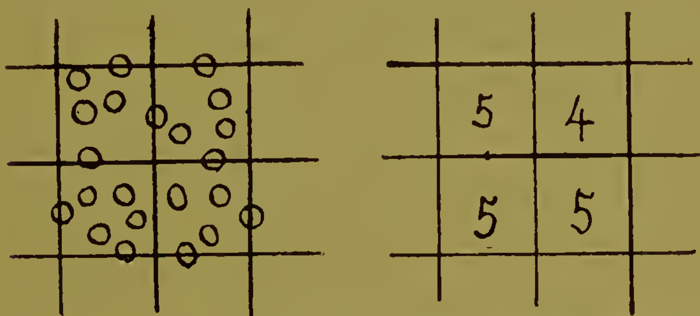

FIG. 28.—Counting Red Corpuscles which overlap the Dividing Lines.

Having counted eight big squares, the figures might be:

100	92	110	101	Total 800.
96	101	95	105	

The average is in this case 100 to the big square, or $\frac{100}{16}$ to the small square. But the volume of fluid over each small square is $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cubic millimetre (p. 52).

$\therefore$ in $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cubic millimetre of 200 times diluted blood there were $\frac{100}{16}$ red corpuscles.

$\therefore$ in 1 cubic millimetre of 200 times diluted blood there were $\frac{100}{16} \times \frac{20 \times 20 \times 10}{1}$ red corpuscles.

$\therefore$ in 1 cubic millimetre of original blood there were $\frac{100}{16} \times \frac{20 \times 20 \times 10}{1} \times \frac{200}{1}$ red corpuscles.

$=$ 5,000,000 red corpuscles per cubic millimetre.

It will thus be seen that, with a dilution of 1 in 200, normal blood containing 5,000,000 red corpuscles per cubic millimetre gives 100 to the big square. Hence, the average number in a big square, the dilution being 1 in 200, gives the percentage of red corpuscles without further calculation.

Importance of counting the Red Corpuscles.—Less can be learned from the number of red corpuscles alone than from the number of red corpuscles and the amount of hæmoglobin at the same time (p. 62). But apart from the hæmoglobin, a count of the red corpuscles may be by itself valuable in the following ways:

(1) In certain persons they may be diminished, though the face be red. Conversely, they may not be diminished, though the face be pale. The

colour of the face may be deceptive, because of vaso-motor nerve influences.

(2) In congenital heart disease (morbus cæruleus) the red corpuscles are frequently much increased; in some cases to 7,000,000 per cubic millimetre; in a few to double this. A similar but less marked increase occurs in those cyanosed from other causes, such as fibroid lung or mitral stenosis.

(3) Those who live at high altitudes usually have more than 5,000,000 per cubic millimetre.

(4) As an anæmic patient improves, the red corpuscles increase more rapidly than does the hæmoglobin. Hence, enumeration of the red corpuscles from week to week affords a valuable means of gauging the effect of treatment.

To count the White Corpuscles.—Proceed in an exactly similar manner with the other pipette. The bore being larger, a bigger drop of blood is required. Care is needed to prevent the fluid running out. Fill to the mark 0·5 with blood; dilute with Thoma's fluid to the mark 11. The dilution is 1 in 20; use the same counting chamber, with the same precautions. In normal blood, diluted 20 times, there are seldom more than three leucocytes in each *large* square, and often none at all in some. Count sixteen large squares; suppose there are a total of 20 leucocytes, the average in one large square would be $\frac{20}{16}$, and in one small square $\frac{20}{16 \times 16}$.

$\therefore$ in $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$ cubic millimetre of 20 times diluted blood there are $\frac{20}{16 \times 16}$ leucocytes.

$\therefore$ in 1 cubic millimetre of 20 times diluted blood there are $\frac{20}{16 \times 16} \times \frac{20 \times 20 \times 10}{1}$ leucocytes.

$\therefore$ in 1 cubic millimetre of original blood there are $\frac{20}{16 \times 16} \times \frac{20 \times 20 \times 10}{1} \times \frac{20}{1}$ leucocytes.

$= 6{,}250$ leucocytes per cubic millimetre.

Importance of counting the White Corpuscles.—Leucocytosis—*i.e.*, an increase in the total number of leucocytes — often accompanies deep-seated suppuration, and may afford valuable help in diagnosis. When doubt exists between pleural effusion and empyema, leucocytosis to 20,000 is in favour of the latter. In a doubtful case of appendicular abscess, leucocytosis to 20,000 or more may turn the scale in favour of laparotomy.

In lobar pneumonia there is often leucocytosis up to 30,000 or more.

In typhoid fever and malaria there is no leucocytosis. In many cases in which obscure pyrexia suggested typhoid fever or malaria, leucocytosis to 20,000 has led to the discovery of deep-seated pus, for example in a pyosalpinx or hepatic abscess.

In the diagnosis of the leuchæmias, lymphatic and spleno-medullary, from Hodgkin's disease and splenic anæmia, a leucocyte count is indispensable. In the two last there is no leuco-

cytosis. In lymphatic leuchæmia the leucocytes rise to as many as 150,000 per cubic millimetre, whilst in the spleno-medullary form 200,000 are often found, and even 600,000.

Though 5,000 per cubic millimetre are regarded as 'normal,' they may reach 12,000 in a healthy man after meals. In children even 15,000 could not be called abnormal.

ESTIMATION OF THE HÆMOGLOBIN.

Haldane's Hæmoglobinometer or *Gower's Hæmoglobinometer.*—The older forms of hæmoglobinometer, in

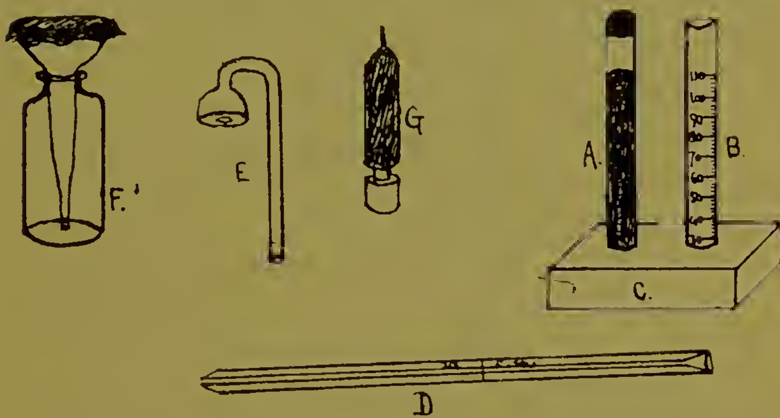

FIG. 29.—Haldane's Hæmoglobinometer.

which tinted glasses are used, are so unsatisfactory that they need not be described. Where coal-gas can be obtained from an ordinary burner, no instrument is better than Haldane's. Where there is no supply of coal-gas, Gower's may be used. Haldane's apparatus (Fig. 29) consists of (1) a sealed tube A, containing a standard solution of carboxyhæmoglobin; (2) a graduated tube B, for diluting the blood in;

(3) an indiarubber stand C, for these tubes; (4) a pipette D, for measuring the blood; (5) a brass U-tube E, for introducing the coal-gas into B; (6) a drop-bottle F, for distilled water; (7) prickers, G. The method is as follows: Drop distilled water into the tube B up to the mark 10. Draw blood into the pipette up to the point indicated on it; expel it into the distilled water in B, without immersing the tip of the pipette. Shake the tube B. The blood is laked at once. [Now wash out the pipette as directed on p. 54.] Fit the short end of the brass U-tube on to an unlighted gas-burner, and push the tube B up over the longer limb until the latter nearly touches the laked blood (Fig. 30). Turn on the gas, and slowly draw away the tube B. The air in its upper part is replaced by coal-gas. On shaking up the laked blood with the gas, the carbon monoxide of the latter converts the hæmoglobin into cherry-red carboxyhæmoglobin. Repeat the introduction of coal-gas if necessary. Now add distilled water drop by drop to the tube B, mixing it up each time, until the tint of the fluid is identical with that of the standard carboxyhæmoglobin in tube A. The graduation now corresponding to the upper level of the fluid in tube B is the percentage of hæmoglobin in the patient's blood, that of normal blood being 100. It is well to make two readings, the first when the tint is just perceptibly darker than the standard, the other when just paler, and to take the mean of the two. With practice the error becomes very small.

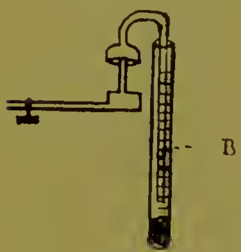

FIG. 30.

Gower's apparatus is very similar in its various parts, but the standard colour-tube contains carminė solution instead of carboxyhæmoglobin, and the laked blood is immediately diluted drop by drop until the tint of mixed oxy- and reduced hæmoglobin is as nearly as possible identical with that of the carmine. The tints can only be made to agree in good daylight, whereas in Haldane's method the solutions are both the same colouring matter, carboxyhæmoglobin, and therefore can be compared in any light, artificial or natural. Carboxyhæmoglobin, moreover, is a very stable compound, and the blood can be kept for examination at any time. Oxyhæmoglobin rapidly changes in shed blood, so that, with Gower's method, comparison must be made at once.

A disadvantage which is common to both instruments is the difficulty of mixing up the fluid in tube B. If the finger be used to close the end, some of the contents, however little, must adhere to the skin; a ground glass stopper would be an improvement.

In country districts it is well to have both a carmine and a carboxyhæmoglobin standard colour-tube for the same apparatus. In the event of coal-gas being unprocurable, the former could be used; in all other cases the latter.

Importance of Estimating the Hæmoglobin.—The severity of most anæmias is better gauged by the amount of hæmoglobin than by the number of red corpuscles. In chlorotic girls the latter may be but little below normal, and yet the hæmoglobin may be very much reduced.

The *colour index* of the blood is the ratio of hæmo-

globin to red corpuscles. Three types of anæmia are based upon it. In healthy blood the colour index is 1; in secondary anæmia directly following hæmorrhage it is still 1; in the chlorotic type it is less than 1; in the pernicious type greater than 1. These characteristics are represented by arbitrary examples in the following table:

	Percentage of Red Corpuscles.	Percentage of Hæmoglobin.	Colour Index.
Healthy person ..	100	100	$\frac{100}{100}$, or 1.
Secondary anæmia	80	80	$\frac{80}{80}$, or 1.
Chlorotic type ..	60	30	$\frac{30}{60}$, or 0·5.
Pernicious type ..	20	30	$\frac{30}{20}$, or 1·5.

Thus, in anæmia immediately following hæmorrhage red corpuscles and hæmoglobin are equally diminished. In chlorotic types the hæmoglobin is diminished more than are the red corpuscles; in pernicious types the red corpuscles are more diminished than is the hæmoglobin.

Most anæmias are of the chlorotic type; for example, chlorosis itself; tuberculous and cancerous cachexias; infective endocarditis and other toxæmias; the later stages of Hodgkin's disease, the leuchæmias, splenic anæmia.

To the pernicious type belong pernicious anæmia, and some cases of ankylostomiasis. Without estimations of both red corpuscles and hæmoglobin and a calculation of the colour index, some cases of infective endocarditis or carcinoma of the stomach may be wrongly diagnosed as pernicious anæmia.

The Preparation and Examination of Blood-Films.

The least wasteful way of storing the various stains is to use half a dozen small cylindrical glass jars (Fig. 31), 3 inches high, $1\frac{1}{4}$ inches inside diameter, with ground stoppers. Films can be immersed bodily in these, and the same stain used again and again.

Use cover-glasses $1\frac{1}{2}$ inches $\times \frac{3}{4}$ inch of No. 1 thickness; others are too thick for the $\frac{1}{12}$ inch objective. Remove all grease from them to insure good films. By the following method it is easy to clean a large number at the same time; they may then be stored for future use. Fill a large evaporating basin or small enamelled saucepan $1\frac{1}{2}$ inches deep with 10 per cent. solution of commercial chromic acid. Boil it. Drop the cover-slips into it *one by one*, and allow them to simmer gently for thirty minutes. Wash away the chromic acid by rinsing repeatedly with tap water, until no trace of yellow remains between any two of the cover-slips. Pour off as much water as possible; rinse the slips with methylated spirit; repeat two or three times with fresh spirit; rinse with absolute alcohol to remove the spirit. With clean forceps transfer each cover-slip separately to fresh absolute alcohol in a glass jar. Finally, pour off the absolute alcohol from this jar and replace it with fresh; the slips are ready for use. When required, pick one out with clean forceps, and pass it through a spirit flame. The alcohol burns off,

FIG. 31.—Cover-glass Bottle.

leaving the glass clean and dry. Should it splinter, the alcohol is not quite anhydrous. Fill the jar with fresh, and the splintering will cease.

To make a film, a good plan is the following: Cut a cigarette-paper at right angles to its length into strips the same width as the slips. Prick the ear; wipe away the first drop of blood; when the second is as large as a big pin's head, smear the ungummed end of a strip over its summit. Holding the other end, lay the smeared surface upon a cover-glass, and draw it steadily along (Fig. 32). With slight practice a perfectly smooth film can be made, the thickness being varied by taking more or less blood, and by altering the angle at which the paper is held to the glass. The blood should dry almost as quickly as it is spread. It is too thick if it stand in slow-drying pools. Use a fresh strip for each film; never smear the cover-glass over twice. If preferred, the film may be made upon a slide instead of on a cover-slip, but the objections are:

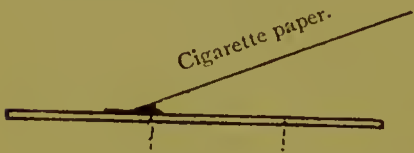

FIG. 32.—Cigarette-paper Method of making Blood Film.

1. The thickness of Canada balsam and cover-slip over it may hinder the use of $\frac{1}{12}$ inch objective.

2. Larger bottles are needed for the stains, unless the latter are poured on to the film, and thus wasted.

Unless carefully fixed, the corpuscles wash off the glass in the process of staining.

METHODS OF FIXING FILMS.—Two are of chief importance:

1. *In Absolute Alcohol and Ether.*—This requires no

special apparatus, and is convenient in private practice. Fill three-quarters of one of the glass jars with absolute alcohol and sulphuric ether, equal parts. Drop in the films when dry, and leave them till next day. An hour is said to be enough, but twenty-four are better. The ether dissolves out the fat from the corpuscles. For ordinary methods of staining this method is excellent.

2. *By Heat.*—A hot-air oven with a good gas regulator is required. For certain stains fixing by heat is essential, but it is inconvenient in private practice. Fix for two hours at a temperature of 120° C., or for half an hour at 140° C.

If there be haste, hold the film in the fingers over a Bunsen burner as hot as can be borne for thirty seconds, and then stain. Fixing is imperfect, but sufficient for many purposes.

METHODS OF STAINING FILMS.—1. *Delafield's Hæmatoxylin and Aqueous Eosine.*—These, after alcohol and ether fixation, stain with certainty, and possess the great advantage that ordinary tap water can be used for washing them. Many other stains require distilled water, and even then are apt to fail unaccountably.

Delafield's Hæmatoxylin.—To 400 c.c. of a saturated aqueous solution of ammonia alum add 4 grammes of hæmatoxylin dissolved in 25 c.c. of absolute alcohol. Leave the solution exposed to the light and air in an unstoppered bottle for three or four days; filter, and add to the filtrate 100 c.c. of glycerine and 100 c.c. of methyl alcohol. Allow the solution to stand in the light until it is a dark colour, refilter, and preserve in a stoppered bottle.

Eosine Solution.

Eosine (aqueous)	1 gramme
Distilled water	100 c.c.

Keep glass jars (Fig. 31) three parts full of each solution, filtering occasionally.

Fix films by absolute alcohol and ether; wash with tap water to remove alcohol and ether; blot with filter-paper; immerse in hæmatoxylin for five minutes; wash thoroughly in running tap water: the slight alkalinity of the latter serves to 'blue' the hæmatoxylin; blot; immerse in eosine for thirty seconds; wash again in running tap water; dry thoroughly with filter-paper; mount in Canada balsam.

Leucocytes: nuclei, blue; protoplasm, purplish or pink; eosinophile granules, bright red.

Red corpuscles: pink; their nuclei, if present, blue.

2. *Methylene Blue and Eosine.*

(*a*) Alcoholic solutions for films fixed by heat:

Alcoholic Eosine.

Eosine	0·5 gramme	
Distilled water	30	c.c.
Absolute alcohol	70	,,

Alcoholic Methylene Blue.

Methylene blue	1·5 grammes	
Rectified spirit	100	c.c.

Allow to stand for twenty-four hours, shaking vigorously from time to time. Filter. Stain in the eosine first, for one minute; wash in distilled water; blot; stain in methylene blue for five minutes; wash in distilled water; examine in water before mounting. If under-

stained with blue, repeat the latter; if over- or under-stained with eosine, do not restain; take another film.

(*b*) Aqueous solutions, for films fixed in alcohol and ether:

Watery Eosine (see p. 66).

Watery Methylene Blue.

Methylene blue	1·5 grammes
Distilled water	100 c.c.

Shake up daily for two weeks. Filter.

Stain the film in eosine for one minute; wash in distilled water; blot; stain in methylene blue for five minutes; wash in distilled water; examine under low power; restain if necessary; dry with filter-paper; mount in Canada balsam.

These two methods are uncertain, though when successful they are pretty.

Red corpuscles: protoplasm, pink; nuclei, if present, deep blue.

Lymphocytes, large and small: nuclei, dark blue; protoplasm, paler blue.

Polymorphonucleated cells: nuclei, blue; protoplasm, pink, with fine granules.

Eosinophile granules: bright red.

Basophile granules: deep blue.

For red corpuscles, the aqueous solutions after fixing in alcohol and ether are best; for leucocytes, heat fixation and alcoholic solutions. If the methylene blue stain faintly, try warming it gently.

3. *Jenner's Stain:*

Crystalline methylene blue and eosine	0·5 gramme
Methyl alcohol	100 c.c.

This has the advantage of fixing the film and staining it at the same time; though extremely rapid, it is uncertain, and tap water cannot be used for washing. Drop the film as soon as dry into the jar of stain for three minutes. Wash in *distilled* water till pink; dry with filter-paper; mount in Canada balsam.

Red corpuscles: terra-cotta coloured; nuclei, if present, blue.

Large and small lymphocytes: pale blue nuclei, dark blue protoplasm; basophile granules, violet.

Polymorphonucleated cells: nuclei, blue; protoplasm, pink, with small red granules.

Eosinophile cells: nuclei, blue; protoplasm, pink, with large red granules.

Bacteria, filaria, and malaria parasites: blue.

4. *Ehrlich's Triple Stain:*

Saturated solution of orange G.	13·14 c.c.
Saturated solution of acid fuchsine ..	6·7 ,,
Distilled water	15 ,,
Absolute alcohol	15 ,,
Saturated solution of methyl green ..	12·5 ,,
Absolute alcohol	10 ,,
Glycerine	10 ,,

Measure off the ingredients in the order named. The mixture may be used at once; but to make each of the saturated solutions it contains requires several days. The stain keeps well, but whereas one batch may stain excellently, the next may fail entirely.

Films must be fixed by heat. Immerse in the stain for one to five minutes; the time varies with different bottles of stain and with the length of time the films have been fixed by heating. Wash with *distilled* water; dry with filter-paper; mount in Canada balsam.

Red corpuscles: pale orange; if overheated, pallid; if underheated, brown. Nuclei, if present, sepia.

Lymphocytes: nuclei, pale or dark blue; protoplasm, pink; no granules.

Polymorphonucleated cells: nuclei, blue; protoplasm, minute purple granules in a pink background.

Eosinophile cells: nuclei, blue; protoplasm, pink; large granules, burnt sienna coloured.

Basophile granules: unstained, clear white spots.

EXAMINATION OF THE FILM.—This may be made with $\frac{1}{6}$ inch objective, but is far better with $\frac{1}{12}$ inch oil-immersion lens, a substage condenser, and a mechanical stage. Select that part of the film in which the corpuscles do not overlap. A differential leucocyte count may be made at the same time. Look for the following points:

CHANGES IN THE RED CORPUSCLES.—In a well-fixed film of normal blood these are smooth in outline, quite round, of approximately equal sizes, and stained rather less at the centre than at the periphery. If the cells are crenated or run together into batches so as to compress one another into polygonal shapes (Fig. 33), the fault is in the film making.

In severe anæmias red corpuscles may vary—

1. *In Size.*—A normal red corpuscle, 7 μ in diameter, is termed a *normocyte*; a smaller is a *microcyte*; a larger a *megalocyte*.

2. *In Shape.*—Instead of round, they may be pear-shaped, oval, fiddle-shaped, or ovoid; such corpuscles are termed *poikilocytes*, and the condition poikilocytosis.

3. *In Staining Capacity.*—A normocyte should stain throughout. When hæmoglobin is very deficient, the corpuscles may resemble quoits, a thin rim of red surrounding a colourless interior.

4. *In the Presence of Nuclei.*—These are best brought out by Jenner's stain, or by eosine and methylene blue. The nucleated red corpuscles have a deep blue nucleus and a rim of pink protoplasm, which readily distinguishes them from the lymphocytes, in which both nuclei and protoplasm are blue. A nucleated

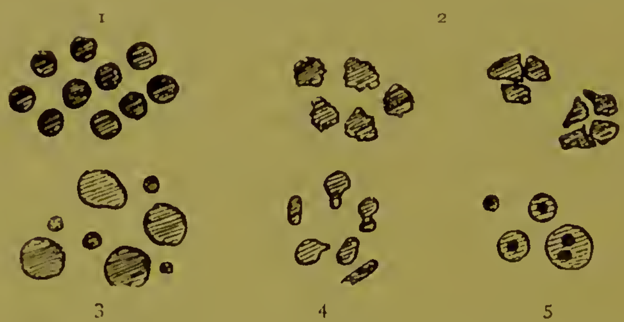

FIG. 33.—Red Blood Corpuscles.

1, Normocytes; 2, badly fixed corpuscles; 3, micro and megalocytes; 4, poikilocytes; 5, nucleated red corpuscles.

normal-sized red corpuscle is termed a *normoblast;* a nucleated small corpuscle a *microblast;* a large one a *megaloblast.* Occasionally more than one nucleus is present in a still larger cell, termed a *gigantoblast.*

Importance of the Changes in the Red Corpuscles.—Poikilocytosis, alterations in size, and presence of nucleated red corpuscles may occur in *any* severe anæmia. Though seen most typically in pernicious anæmia, these changes are not pathognomonic. Since, however, those corpuscles stain best which contain most hæmoglobin, and since the colour-

index is above par in pernicious anæmia, below par in chlorotic forms, good staining capacity associated with nucleated red corpuscles and alterations in shape and size are points favouring a diagnosis of pernicious anæmia; conversely, 'quoit' forms are in favour of some chlorotic form.

A high proportion of nucleated red corpuscles indicates very severe anæmia, and a less good prognosis; conversely, if the proportion of nucleated forms diminishes, the patient is improving. The proportion of nucleated red corpuscles is conveniently expressed by the number seen in counting 100 leucocytes in the film.

DIFFERENTIAL LEUCOCYTE COUNT.—A mechanical stage is essential. Omit the thicker parts of the film; in the remainder examine the leucocytes consecutively, allotting each to its appropriate class. Begin at one end of the film, say the top right-hand corner, and screw it continuously past the eye until the left edge of the thin part is reached; then, watching through the microscope, screw it down one field's breadth—*i.e.*, until that corpuscle which was the top one in the field becomes the bottom one—and then count the leucocytes continuously back again until the right edge is reached; turn the film down another field's breadth, and count it transversely across to the left edge again; and so on, backwards and forwards, until the required number of corpuscles, say 250, has been enumerated. In normal blood these will fall into four main groups, to which various names have been given (Fig. 34). They are:

1. *Small Lymphocytes.*—Slightly larger than red

corpuscles, variable in size, characterized by a comparatively large, deep-staining spherical nucleus, and a rim of protoplasm which is sometimes very narrow, sometimes considerable. These constitute from 20 per cent. to 30 per cent. of leucocytes in health.

2. *Large Lymphocytes.* — Also termed 'hyaline'; much larger cells, with notched or kidney-shaped nucleus, and much pale-staining clear protoplasm. These constitute from 2 per cent. to 10 per cent. in

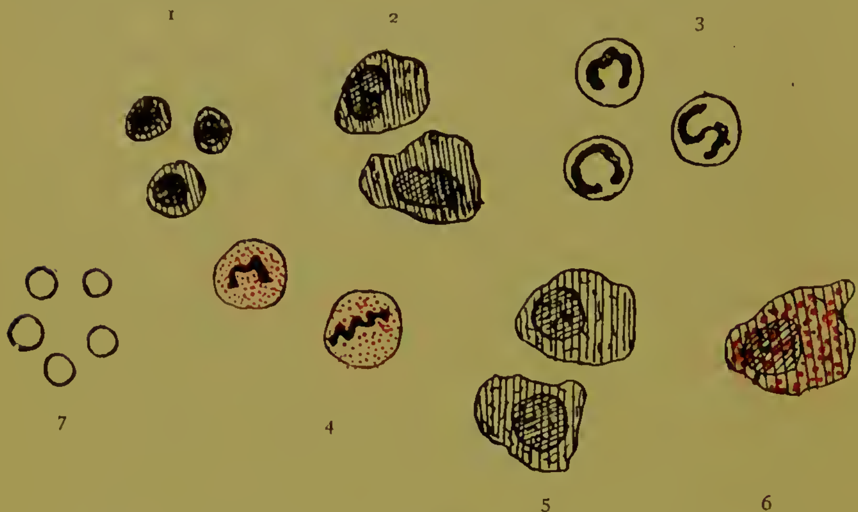

FIG. 34.—Leucocytes.

1, Small lymphocytes; 2, large lymphocytes; 3, polymorphonucleated cells; 4, coarsely granular eosinophile cells; 5 and 6, myelocytes; 7, red corpuscles.

health. Some cells are intermediate between typical small and typical large lymphocytes, classed with the former by some, by others with the latter. Most class all such intermediate cells with the small lymphocytes, reserving the term 'large lymphocytes' for the typical large cells only.

3. *Polymorphonucleated Cells.* — Somewhat smaller than large lymphocytes; usually circular, with a well-

defined nucleus variously lobed and twisted. The name of this cell is derived from this characteristic of the nucleus, which may be horse-shoe shaped, or twisted like the letters Z, S, E. Other names used are 'neutrophile' and 'finely granular oxyphile.' The protoplasm is abundant, and shows minute granules; these cannot be mistaken for the coarse granules of the next variety when once the latter have been seen. The polymorphonucleated cells constitute anything between 60 per cent. and 80 per cent. in health.

4. *Coarsely Granular Eosinophile Cells, or, shortly, 'Eosinophiles.'* — Slightly larger than polymorpho-nuclears, with a similar twisted nucleus, and protoplasm which is crowded with unmistakable coarse granules. They vary from 0·5 per cent. to 5 per cent. in health.

Two other forms of leucocytes are found under certain pathological conditions, namely:

5, 6. *Myelocytes.*—Large cells with round or oval pale-staining nuclei, and much protoplasm. They differ from the large lymphocytes in possessing fine granules and streaks. In certain myelocytes the granules stain bright red with Ehrlich's triple stain; and these eosinophile myelocytes might be mistaken for coarsely granular eosinophiles were it not that the nuclei of the latter are lobed, whilst that of each myelocyte is large and rounded.

Basophile Cells.—Not much larger than red corpuscles, with a round nucleus, and coarse granules which are best stained with the following dye:

Saturated alcoholic solution of dahlia, filtered	50 c.c.
Glacial acetic acid	10 ,,
Distilled water	100 ,,

Fix films by heat. Leave them in the stain for twenty-four hours. Wash in distilled water; dry with filter-paper; mount in Canada balsam.

Importance of the Differential Leucocyte Count.—So variable are the proportions of the white corpuscles in health that too much stress must not be laid upon comparatively slight increases in any particular variety.

The small lymphocytes are increased:

Up to 35 per cent. or more in many healthy infants.

Up to 40 per cent. or 50 per cent. in children suffering from congenital syphilis.

Up to 50 per cent. or 60 per cent. in children suffering from whooping-cough.

Up to 40 per cent. in persons suffering from urticaria.

In typhoid fever, especially in mild attacks; in case of obscure pyrexia, without leucocytosis, an increase of small lymphocytes up to 35 per cent. or 40 per cent. favours a diagnosis of enterica, and in the tropics may be a valuable means of excluding malaria.

In lymphatic leuchæmia, in which there is leucocytosis to 50,000 or 150,000, and a relative increase of small lymphocytes even up to 95 per cent.

The large lymphocytes are increased:

In malaria, in which, as a rule, the total number of leucocytes is under 5,000 per cubic millimetre, whilst the large lymphocytes may be 15 per cent.

to 30 per cent., affording a valuable means of diagnosis between malaria and enterica.

The polymorphonucleated cells are increased:

In certain stages of lobar pneumonia, with leucocytosis.

In the leucocytosis of deep-seated suppuration, such as empyema, appendicular abscess, pyosalpinx. Leucocytosis and a rise of polymorphonuclears to over 80 per cent. may often help to exclude enterica and suggest deep-seated pus.

The coarsely granular eosinophile cells are increased:

In many cases of true asthma, when they may help to exclude diagnosis of thoracic aneurism or mediastinal growth. The increase may be to anything between 6 per cent. and 60 per cent.

In certain parasitic affections, notably in ankylostomiasis; in *Bilharzia hæmatobia;* in trichinosis; in filariasis; and in patients suffering from tape-worms. The eosinophilia may be slight, up to 10 per cent., for example; it is often between 10 per cent. and 20 per cent., and occasionally reaches 50 per cent. Nothing under 5 per cent. can be termed eosinophilia.

In certain skin diseases, particularly true pemphigus, and hydroa herpetiformis, where from 6 per cent. to 20 per cent. are found. In other skin diseases, such as psoriasis or eczema, the majority of patients show no eosinophilia, though cases with increase to 5 per cent. or more occur occasionally.

The myelocytes are present in numbers in one disease only — namely, spleno-medullary leu-

chæmia, where they may constitute 45 per cent. of all the white corpuscles. They are not, however, distinctive of this condition; smaller numbers are found in splenic anæmia and Hodgkin's disease; and a few occur in almost any anæmia that is severe.

The basophile cells are not pathognomonic of any disease. Even in health a few may be found up to 0·5 per cent. In spleno-medullary leuchæmia they may rise to 5 per cent., but what importance attaches to this is not yet known.

The following notes on the main characteristics of the primary anæmias may be useful.

Spleno-medullary leuchæmia: Hæmoglobin index 1 or less; big spleen; no big glands; leucocytosis, possibly to 400,000; myelocytes up to 45 per cent.

Lymphatic leuchæmia: Hæmoglobin index 1 or less; big spleen; big glands; leucocytosis up to 150,000; small lymphocytes may be 95 per cent.

Hodgkin's disease: Hæmoglobin index 1 or less; big spleen; big glands; no leucocytosis; leucocytes often in normal proportions.

Splenic anæmia: hæmoglobin index 1 or less; big spleen; no big glands; no leucocytosis.

Pernicious anæmia: Hæmoglobin index greater than 1; no big spleen; no big glands; no leucocytosis; white corpuscles in normal proportions.

Chlorosis: Hæmoglobin index less than 1; no big spleen; no big glands; no leucocytosis; white corpuscles in normal proportions.

(*N.B.*—In all the above, the red corpuscles may

vary in size and shape, and nucleated forms may be present.)

The Presence of Parasites—*Malaria.*—Films may be fixed and stained in any of the ways already described. It is best, however, to look for parasites in a fresh drop of blood received upon the centre of a cover-slip, lowered gently on to a microscope slide, and allowed to spread out evenly and thin. Should the corpuscles run into rouleaux, or be more than one cell deep, make another preparation. Ring the edge with vaseline to prevent evaporation, and examine the fresh blood with $\frac{1}{12}$ inch oil-immersion lens and a mechanical stage. Search the corpuscles as in making a differential leucocyte count, and do not say that hæmatozoa are absent unless none have been found after a careful search for half an hour. After quinine has been given none will be present. In the tertian form the earliest stage (Fig. 35) is a pale amœboid spherule, situated excentrically in a red corpuscle, not filling the cell, and without pigment. Later, the spherule has enlarged so as nearly to fill the cell, and through it are scattered fine granules of dark pigment. These two stages are those most usually seen. The last stage, that of sporulation, corresponds to the onset of the rigor, and is rarely seen without a warm stage and careful watching. The corpuscle is transformed into a rosette of 12 or 15 spores, radiating from a central collection of fine pigment granules.

Fig. 35.—Malaria Parasites.

In the case of the quartan parasite similar changes are seen, but the parasite is smaller, has fewer, larger, darker pigment granules, and in the rosette stage has fewer spores.

In either of these forms, when the blood has been shed an hour or more, flagellated forms may appear (Fig. 35,[4]). Apparently these never occur in circulating blood; the flagellæ seem to grow out from the parasite, and, by their lashing movements, swirl the surrounding corpuscles about.

In æstivo-autumnal malaria a crescentic form occurs (Fig. 35,[5]), with pigment granules at its centre, and the ghost of a red corpuscle attached to its concave side.

Filaria Sanguinis Hominis.—This is only found in persons who have been in the tropics. The mature worm occupies the pelvic lymphatics; embryos alone occur in the blood, and in the common form, *Filaria nocturna*, are present in the peripheral circulation at night only. In the daytime they are in the visceral bloodvessels. Search for them after 9 p.m. In a rarer form, *Filaria diurna*, they occur in the peripheral blood only in the daytime. In rarer cases still, *Filaria perstans*, they are found both day and night. Fix and stain films in the ordinary way. The embryo, if present, is readily seen. It is as long as fifty red corpuscles (Fig. 36), though only as wide as one.

FIG. 36.—Filaria Embryo in Blood.

Spirillum of Relapsing Fever (Fig. 37).—This is most

readily recognised in a drop of fresh blood, as in examining for malaria parasites. The organism is motile, but disturbs the surrounding red cells more by its twisting movements than by its actual locomotion. Though, when straightened out, six times as long as the diameter of a red corpuscle, it is a mere thread in width, so that a high power is needed to discover it. Few in numbers at the beginning of a febrile paroxysm, they increase during the fever, and disappear in the intervals of apyrexia. They may be found in blood taken from one part of the body, and not in that from another part taken at the same time.

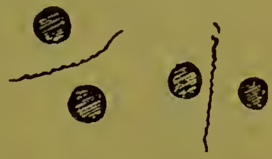

FIG. 37.—Spirillum of Relapsing Fever.

ESTIMATION OF THE SPECIFIC GRAVITY.

Chloroform (specific gravity 1480), benzol (specific gravity 888), a sensitive urinometer graduated from 1020 to 1080, and a suitable glass cylinder are required. Mix chloroform and benzol until the specific gravity of the mixture is 1058, which is that of normal blood. Prick the lobule of the ear; draw up blood into a hæmoglobinometer pipette, taking care to avoid an air bubble; expel a drop into the mixture. Should it float, the specific gravity of the mixture is higher than that of the blood. Reduce it by adding more benzol. Should the blood sink, raise the specific gravity of the mixture by adding chloroform. Should the drop have an air bubble in it, or should it adhere to the side of the cylinder, take a fresh drop. When the blood drop neither sinks nor floats, the specific gravity of the

mixture is the same as that of the blood, and may be read off on the urinometer (Fig. 38). There is a certain error, constant for the same urinometer, but sometimes as much as 5 degrees of specific gravity, because the instrument is graduated for *urine*, the surface tension of which is different to that of the chloroform and benzol mixture.

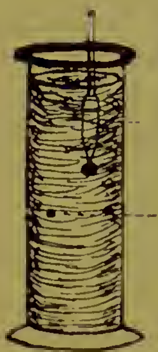

Fig. 38.—Specific Gravity of Blood.

Importance of Estimating Specific Gravity of Blood.—The specific gravity rises when the blood becomes concentrated, and therefore affords a means of diagnosis between collapse and shock. Collapse follows great loss of fluid, the blood becomes concentrated, the specific gravity rises; treatment by transfusion is indicated. In shock there has been no loss of fluid, the specific gravity is not raised, transfusion is not indicated.

The specific gravity also affords a valuable check upon the hæmoglobin estimations. Specific gravity and hæmoglobin rise and fall together; approximately:

A sp. gr. of	1035	corresponds to	30	per cent.	of	hæmoglobin
,, ,,	1040	,, ,,	45	,,	,,	,,
,, ,,	1050	,, ,,	65	,,	,,	,,
,, ,,	1055	,, ,,	75	,,	,,	,,
,, ,,	1060	,, ,,	100	,,	,,	,,

Lipæmia.—In certain cases of diabetes mellitus the blood becomes pale and 'milky,' a condition which is in some cases due to fine globules of fat, in others to

fine particles of a precipitated proteid circulating in the blood. A yellow colour of the retinal blood-vessels may suggest the onset of 'lipæmia'; microscopic examination of a drop of blood may confirm it. Fat globules will stain black on the addition of 1 per cent. solution of osmic acid. Proteid particles do not stain black, but appear as bright refractile spheres, smaller than the red corpuscles. Either indicate the imminence of coma, but in certain cases early recognition has led to the administration of large quantities of carbonate of soda in time to avert the fatal issue.

WIDAL'S REACTION FOR TYPHOID FEVER.—The 'clumping reaction' is not so much a vital as a physical phenomenon. The test may be made with dead cultures, although, where possible, it is best to send the patient's blood serum to a bacteriological laboratory for testing with living cultures.

To obtain the serum a glass pipette (Fig. 39) is

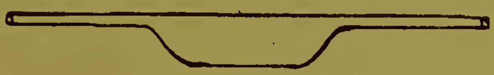

FIG. 39.—Widal Tube.

needed, consisting of a bulb drawn into a capillary tube at either end, and sterilized in boiling water. Cleanse the lobule of the patient's ear by washing with boiled water, prick the lobule deeply with a sterilized needle; from the little pool which collects suck blood into one end of the pipette until the bulb is a quarter full. Take great care not to let any run down into the further end of the bulb. Holding the pipette horizontal, seal off both ends in a spirit flame; leave it lying horizontally for an hour. The blood will have

completely clotted. Now turn the blood-containing end vertically up (Fig. 40); the serum from the clot will run down into the clean end, and fill the capillary tube. Unless the serum be obtained free from corpuscles, bacteria subsequently mixed with it cannot be properly watched. The pipette is now ready to be sent to a special laboratory by post. If, on the other hand, the serum is to be tested with a dead culture, proceed as follows:

Break the end off the serum end of the pipette, and receive the serum in a clean watch-glass. Take a platinum wire loopful and transfer it to a clean cover-slip, and on to the same cover-slip put nine separate loopfuls of 0·6 per cent. salt solution. Mix thoroughly. The serum dilution is 1 in 10. Transfer a loopful of this diluted serum to another cover-slip, and add to it nine separate loopfuls of salt solution. Mix thoroughly. The second serum dilution is 1 in 100. Now put a loopful of the dead typhoid bacillus culture on to each of two clean cover-slips; to the first add a loopful of the serum diluted 1 in 10, making a dilution of 1 in 20; to the second, a loopful of the serum diluted 1 in 100, making a dilution of 1 in 200. Invert each cover-slip over a hanging drop-chamber, the edge having been vaselined to prevent evaporation, and watch the bacilli in the hanging drop under $\frac{1}{6}$ inch power. If the test be positive, the bacilli will adhere to one

FIG. 40.

another in batches or 'clumps' instead of being evenly distributed. The test gives positive proof of past or present typhoid fever if the clumping occur within half an hour with a serum dilution of 1 in 200. If the clumping take place with a dilution of 1 in 20, and not with 1 in 200, typhoid fever may or may not be present. The diagnosis is greatly assisted should there be at the same time absence of leucocytosis and a relative increase in small lymphocytes in the blood. Absence of clumping with a dilution of 1 in 20 almost excludes typhoid fever, unless it be within the first week of the illness.

CHAPTER III.

EXAMINATION OF THE SPUTUM.

I. NAKED-EYE CHARACTERS.

Certain macroscopic appearances of the sputum are typical.

In bronchitis it is at first small in amount, and frothy from admixture of air with serous exudation. Small, clear, sticky pellets of mucus occur in the froth. Later the expectoration, though still frothy upon the surface, has a thick, opaque, yellowish deposit of muco-pus.

In phthisis with cavitation nummular sputum is distinctive. Heavy airless lumps of muco-pus are coughed up. If expectorated on to a dry surface each flattens out into a yellowish-white circular meniscus, which does not coalesce with its neighbours; hence called 'nummular,' or coin-like. If expectorated into carbolic lotion or other fluid, each more or less spherical, airless mass sinks to the bottom, but remains distinct from its neighbours. Streaks of red blood occur in the earlier stages; later, blood-clots resembling in shape the lung cavities. It must not be forgotten that streaks and clots of blood result also

from adenoids, mitral stenosis, fibroid lung, whooping-cough, severe bronchitis.

In fibroid lung with bronchiectasis the amount of sputum may be great, up to as much as 1 pint or more in the twenty-four hours. It often smells very foul. Usually expectoration occurs at comparatively long intervals, when a large quantity is coughed up at a time. Received into a specimen-glass, it settles into three layers (Fig. 41): upon the surface, frothy serum; at the bottom, a thick sediment of muco-pus; between, clear serous fluid.

In lobar pneumonia the sputum is termed 'rusty.' This name applies to its usual colour, a dull red-brown. The colour is not constant, for it is due to blood, and may be any hue that a bruise may be, from bright red to dull red, brown, bluish, green, or yellow. The chief characteristic of pneumonic sputum is its viscosity. So glairy is it that, if expectorated into a dry porringer, the latter may be turned upside down, and yet the sputum will only very slowly slide along its surface. Such glairy sputum, whatever be its colour, is almost pathognomonic of lobar pneumonia.

FIG. 41.—Sputum in Bronchiectasis.

In plastic bronchitis, a rare condition, casts of the bronchioles occur. Similar casts may be found in cases of diphtheria in which membranous exudate has extended into the tubes.

Anchovy-sauce-coloured expectoration is rare, but is almost pathognomonic of hepatic abscess ruptured into the lung.

No other types of sputum can be called characteristic. In infarction of the lung it is said to resemble 'prune-juice,' in growth of the lung 'red-currant jelly.' Both descriptions are fanciful, and not pathognomonic. Morning expectoration of thick phlegm stained black by smoke and dust occurs in healthy persons who live in towns or smoky atmospheres.

II. MICROSCOPICAL CHARACTERS.

A. Sputum in Phthisis.

It is in cases of phthisis, suspected or certain, that investigations of the sputa are so important. The following points should be attended to:

1. The amount. Large quantities indicate cavitation or bronchiectasis.
2. The character. Nummuli indicate cavitation.
3. The presence of blood. Streaks in early phthisis; clots when there is cavitation.
4. The presence of elastic fibres.
5. The presence of tubercle bacilli, and whether few, many, or very many.
6. The presence of other bacteria, particularly staphylococci and streptococci.
7. The proportion of pus cells to mucus.

Elastic Fibres.—Take 1 inch of the nummular sputum in a test-tube, add 1 inch of 10 per cent. caustic soda, and boil for five minutes. All the organized elements except the elastic fibres are destroyed. Pour the swollen gelatinous residue into 3 inches of water in a specimen-glass, stir well, and allow to settle. If necessary, centrifugalize the de-

posit. The elastic fibres may be made more evident by adding some magenta solution,

Magenta Stain.

Fuchsine	0·5 gramme
Distilled water	200 c.c.

which stains them dark purple, almost black. Transfer some of the deposit to a drop of water upon a slide, and examine under a low power. Elastic fibres (Fig. 42) are sharply defined, double-contoured threads, sometimes single, sometimes branched, often spirally twisted at their free ends, and occasionally arranged

FIG. 42.—Elastic Fibres from Lung.

in a meshwork corresponding in outline to a pulmonary air vesicle.

Importance.—They indicate a serious condition of active lung disintegration, such as occurs in advancing phthisis, gangrene, or pulmonary abscess.

TUBERCLE BACILLI.—Make a film. It is essential that the sample taken for this purpose should be from the interior of a nummulus. It is best picked out by a fine pair of forceps, and it should be smeared *fairly thickly* over a clean cover-slip and allowed to dry in the air. Disinfect the forceps with heat. In many cases it is sufficient to make a film in this way without

previous treatment of the sputum, but where tubercle bacilli are few, they may not be found without first breaking up the nummuli as follows:

Into a small beaker put 10 c.c. of the sputum and 20 c.c. of water; add 5 drops of 10 per cent. caustic soda, boil gently for five minutes, stirring well; add 100 c.c. of cold water, stir well, and allow to settle. Centrifugalize the deposit, and proceed to make films. After such treatment bacilli will not adhere to the cover-slip unless the latter be albuminized (seen p. 14).

Having made and dried the film, fix it by passing slowly through a Bunsen flame five times, film upwards, then stain by the *Ziehl-Neelsen method.*

Carbol Fuchsine Stain.

Saturated alcoholic solution of fuchsine	10 c.c.
Carbolic acid crystals	5 grammes
Distilled water to	100 c.c.
Add absolute alcohol	10 ,,

Sulphuric acid: 25 per cent. solution.

Carbol Methylene Blue Stain.

Methylene blue	1·5 grammes
Carbolic acid crystals	5 ,,
Distilled water to	100 c.c.
Add absolute alcohol	10 ,,

In an evaporating basin warm some carbol fuchsine until it begins to steam freely. Do not boil it. Immerse the film in it for five minutes. Remove the film, wash it in tap water, and then immerse it in 25 per cent. sulphuric acid. Its red colour changes to orange yellow, and much of the stain is discharged. In one minute wash off the acid with tap water. The

red colour will reappear. If it be a faint pink, enough stain has been removed; if a darker red, return the film to the sulphuric acid until, on washing with tap water, only a faint pink remains. The tendency is to remove too little stain rather than too much. Now blot with filter-paper and transfer to carbol methylene blue for three minutes; wash in tap water; blot; allow to dry in the air; mount in xylol balsam, and examine under $\frac{1}{12}$ inch oil-immersion lens with a good Abbé condenser.

The principle of the above method is as follows: Tubercle bacilli take up stain with difficulty, but once they are stained it is difficult to unstain them. Cold carbol fuchsine will not stain them, warm will; but at the same time all the other structures are greatly overstained. The sulphuric acid corrects this by removing the fuchsine from everything except the tubercle bacilli. Leprosy bacilli and smegma bacilli are the only other organisms which retain the stain in the same way. Carbol methylene blue counterstains the remaining structures so that tubercle bacilli stand out red in a blue field.

They are too small to be seen easily with $\frac{1}{6}$ inch power. Each measures about 3 μ in length, or less than half the diameter of a red corpuscle, and they are exceedingly thin. They frequently have a beaded appearance, though they are said not to form spores. They may occur singly or in pairs joined end to end (Fig. 43), or in clusters of half a dozen or more.

Importance.—Tubercle bacilli are never adventitious; their presence means tuberculous trouble. It is not possible, however, to say that tubercu-

losis is absent because no organisms have been found. Careful and repeated search may be needed before such negative evidence can be relied on. In acute miliary tuberculosis of the lung they may not be found at all. On the other hand, presence of large numbers is not a hopeless sign. They are evidence of active mischief, but recovery is possible. It must be remembered that numbers may be present one day, few another, so that repeated examinations should be made.

Fig. 43.—Tubercle Bacilli and Pus Cells.

Other Pathogenic Bacteria in Phthisical Sputum.—*Staphylococci.*—Each about 1 μ in diameter, arranged in clusters and irregular clumps (Fig. 47, p. 97).

Streptococci.—Each about 1 μ in diameter, arranged in short or long strings (Fig. 48, p. 98).

Diplococci.—Each about 1 μ in diameter, arranged in pairs, with an unstained halo round each pair (Fig. 50, p. 99).

Importance.—As a general rule, when many tubercle bacilli are present, there are few other organisms, and *vice versâ.* Many of the severe symptoms of phthisis are due to staphylococci and streptococci. When such secondary infection has occurred, cachexia and toxic symptoms increase, pyrexia is more constant, and the prognosis, as a rule, worse. In this respect, the presence of streptococci is more serious than that of staphylococci only. Diplococci have no par-

ticular significance in such cases; they may, indeed, be found in perfectly healthy persons.

NON-PATHOGENIC BACTERIA IN PHTHISICAL SPUTUM.—*Bacteria* of various kinds; their names are not important.

Tetrads or *Sarcinæ*, consisting of cocci arranged in fours, or multiples of four (Fig. 44).

Importance.—These indicate putrefaction in the cavities in the lung, and are therefore a bad sign, though in themselves harmless. They occur in bronchiectasis as well as in phthisis.

FIG. 44.—Tetrads and Pus Cells.

PUS CELLS AND MUCUS IN PHTHISICAL SPUTUM.—The former are chiefly polymorphonucleated leucocytes (see Fig. 34, p. 72), the latter structureless streaks and wisps. Both are stained blue.

Importance.—In the earlier stages of phthisis mucus predominates; later pus cells may be extremely numerous, and indicate suppurating cavities, with consequent liability to lardaceous disease.

In addition to the above constituents of a film, large squamous cells may occur; these are derived from the buccal mucosa, and have no pathological significance.

B. SPUTUM IN OTHER CONDITIONS.

In *lobar pneumonia* the viscid character of the sputum is almost pathognomonic. Microscopical examination is confirmatory, but not essential. Make cover-slip films, allow them to dry, fix them by passing five

times through a Bunsen flame, and stain either with carbol methylene blue (p. 88), or with carbol fuchsine (p. 88), or by Gram's method (p. 96). Examine them under $\frac{1}{12}$ inch oil-immersion lens, using an Abbé's condenser. Lobar pneumonia is caused by more organisms than one. The film will show many pus cells, some red corpuscles, streaks of mucus, probably groups of staphylococci and strings of streptococci, and numbers of one or other of the following bacteria:

Diplococci Pneumoniæ of Fraenkel (Fig. 50, p. 99).—The causative agent in most cases. Each organism is an oval coccus about 1 μ in longest diameter; they occur in pairs, with an unstained halo or capsule round each pair. They stain with carbol fuchsine and with carbol methylene blue. They retain the stain by Gram's method. Their capsule may be stained by the following process:

Capsule Stain.

Dahlia	0·5 gramme
Methyl green (00 crystals)	1·5 grammes
Saturated alcoholic solution of fuchsine	10 c.c.
Distilled water to	200 ,,

Immerse a film in the stain for five minutes; wash gently in distilled water; blot; dry in the air; mount in Canada balsam.

Pneumo-bacilli of Friedländer.—These are found in fewer cases, but are easily mistaken for diplococci in that they are arranged in pairs within a capsule. Each organism is, however, a short rod, and not a coccus; moreover, it will *not* stain by Gram's method. It stains with carbol fuchsine and with carbol methylene blue, and the capsule is brought out by the capsule stain.

Influenza Bacilli.—Exceedingly minute rods, 1·5 μ long by 0·3 μ broad. They are not arranged in pairs, and have no capsule. They stain with carbol fuchsine and with carbol methylene blue, but not by Gram's method.

Importance. — Each of these organisms may occur even in considerable numbers in the sputum of patients who are not suffering from pneumonia. This is particularly true of diplococci, which have been found in healthy expectoration. In pneumonia more stress is to be laid upon the viscidity of the sputum than upon the micro-organisms it contains. At the same time, presence of influenza bacilli in the sputum may render material assistance in the diagnosis of influenzal pneumonia.

Charcot-Leyden Crystals.—These consist of phosphate of spermin; are colourless, of elongated diamond shape, and comparable in size to pus corpuscles (Fig. 45). Spread out a fragment of the fresh sputum on a slide under a cover-glass, and examine under $\frac{1}{6}$ inch power.

FIG. 45.—Charcot-Leyden Crystals and Pus Cells.

Importance.—They are not pathognomonic of any particular affection, nor are they common. They occur in some cases of asthma, but not in all. They have been found in bronchiectasis and in chronic phthisis. Since they have also been obtained from seminal fluid, and from leucocythæmic blood, they are regarded as a product of cell disintegration.

Curschmann's Spirals.—These may be seen in fresh sputum under the low power, and may even be visible to the naked eye. Each consists of a central core, round which is woven a thick spiral mesh-work of delicate fibres (Fig. 46). They have all manner of shapes and sizes.

Importance.—Apparently they are mucoid casts of the finest bronchioles, and may therefore occur in any condition where these are affected. They have been found in broncho-pneumonia, in bronchiectasis with bronchitis, in capillary bronchitis, in asthma. If any significance can be attached to them at all, other than that they indicate affection of the bronchioles, it is in asthma, where their presence perhaps shows that the patient has bronchial asthma as distinct from other forms, such as uræmic or cardiac. Their absence, on the other hand, does not] exclude affection of the bronchioles.

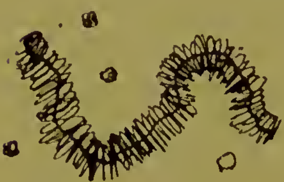
FIG. 46.—Curschmann's Spiral and Pus Cells.

CHAPTER IV.

EXAMINATION OF PUS.

THE chief point to be determined is the variety of organism present. With a pair of fine forceps or a platinum wire, smear out some of the pus in a thin layer upon a cover-slip previously cleansed by the chromic acid process (p. 63); allow the film to dry spontaneously in the air; when quite dry, pass it through a Bunsen or spirit flame five times, thereby 'fixing' by coagulation of the albumin. Stain by one or other of the following methods :

1. *With Carbol Methylene Blue.*—The composition of the stain is given on p. 88. Immerse the film in it for five minutes; wash gently in water for a few seconds; blot with filter-paper; dry in the air; mount in Canada balsam.

2. *With Carbol Fuchsine.*—The composition of the stain is given on p. 88. Immerse the film in it for three minutes; wash vigorously in water for 30 seconds; blot; dry in the air; mount in Canada balsam.

This and the preceding method will stain all the organisms described below except the tubercle bacillus. With carbol fuchsine there is danger of overstaining,

whereas carbol methylene blue is a less energetic dye. In each case the carbolic acid acts as a mordant, and is essential.

3. *By Gram's Method.*—The following solutions are required:

(*a*) *Aniline Water:*

Aniline oil	5 c.c.
Water	100 ,,

Shake up together in a bottle; the water becomes impregnated with the aniline oil, but globules of the latter remain undissolved. The water must therefore be filtered when required for use.

(*b*) *Gentian Violet Stain:*

Saturated solution of gentian violet in absolute alcohol.

(*c*) *Gram's Iodine Solution:*

Iodine	1 gramme
Potassium iodide	2 grammes
Distilled water	300 c.c.

Fill an ordinary watch-glass three parts full of filtered aniline water; add 5 drops of gentian violet solution; immerse the film in the mixture for five minutes. Wash off the free stain with water; transfer the film to Gram's iodine solution in a watch-glass; previously violet, it here becomes a deep slate colour. After one minute, remove it from the iodine, and wash it with alcohol; much of its colour will be discharged until it is a pale gray. Wash it in water again; the film should now regain

a quite pale violet colour. If the tint be at all deep, insufficient stain has been removed, in which case repeat the iodine process; then blot off the water with filter-paper, allow the film to dry in the air, and mount in Canada balsam.

Some organisms retain the gentian violet in spite of subsequent treatment with iodine, others do not; the method is therefore of considerable assistance in diagnosis.

4. *By the Ziehl-Neelsen Method.*—For tubercle bacilli, see p. 88.

5. *By the Capsule Stain.*—For pneumococci and for pneumobacilli, see p. 92.

Examine the stained film with $\frac{1}{12}$ inch oil-immersion lens, using an Abbé's condenser; without the latter, bacteria are difficult to see. Occasionally, and particularly in tuberculous pus, no organisms may be present. More often bacteria will be found, and the following are the microscopical characteristics of the commoner kinds:

STAPHYLOCOCCUS PYOGENES.—Each is spherical, about 1 μ in diameter; it occurs in irregular clusters or masses. Three varieties are described: (*a*) Albus; (*b*) aureus; (*c*) citreus, according to the colour of the cultures on gelatine. Under the microscope all look alike (Fig. 47).

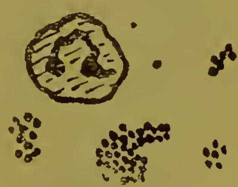

FIG. 47.—Pus Cell and Staphylococci.

It retains the stain by Gram's method.

Importance.—It is of frequent occurrence in subcutaneous abscesses, sputum from lung cavities, cystitis, and, indeed, in almost all forms of suppura-

tion. It is usually much less virulent than other organisms which cause suppuration.

STREPTOCOCCUS PYOGENES.—Each is spherical, about 1 μ in diameter; it forms chains, which may be long or short, the organism being called in the one case *Streptococcus longus*, in the other *Streptococcus brevis* (Fig. 48). There is, however, insufficient evidence to prove the definite existence of more kinds of streptococci than one.

FIG. 48.—Streptococci and Pus Cell.

It retains the stain by Gram's method.

Importance.—A virulent organism in many cases. Not so often found as staphylococcus, but a more serious infection. It may occur in simple abscesses, phthisical sputum, pyorrhœa alveolaris, empyemata, suppurative peritonitis, erysipelas, infective endocarditis, osteomyelitis, septic conditions of the uterus, pyæmia, septicæmia.

GONOCOCCUS.—Each is rather larger than a staphylococcus, and is kidney-shaped rather than spherical; they occur in pairs, but have no capsule, and they are found *inside* the pus corpuscles as well as outside (Fig. 49).

It does not retain the stain by Gram's method.

FIG. 49.—Gonococci and Pus Cells.

Importance. — Most usual in gonorrhœa; its presence in urethral pus may elucidate a doubtful case of urethritis. It dies out, however, in chronic cases. It may also occur in gonorrhœal

ophthalmia, synovitis, abscesses, and infective endocarditis.

Pneumococcus of Fraenkel.—Each is 1 μ in diameter, not quite spherical, owing to a sharp, lancet-shaped projection. They are arranged in pairs, with the points in opposite directions, and each pair is surrounded by a capsule.

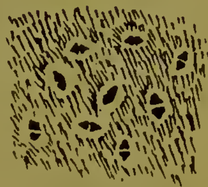

Fig. 50.—Pneumococci.

The cocci retain the stain by Gram's method, but the capsule appears as a colourless halo. For the capsule stain, see p. 92.

Importance.—The chief cause of lobar pneumonia, in the sputum of which it may be found in abundance. It is, however, sometimes found in healthy persons, or in phthisical sputum where there is no pneumonia. It occurs in many cases of empyemata; and more rarely in pneumococcal peritonitis, arthritis, meningitis, infective endocarditis.

Pneumobacillus of Friedländer.—This is difficult to distinguish from the pneumococcus under the microscope, except by the fact that it does not stain by Gram's method. Each bacillus is so small that it is readily mistaken for a coccus. They are, moreover, arranged in pairs within a capsule, which requires special staining (see p. 92).

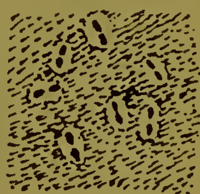

Fig. 51.—Pneumobacilli.

Importance.—A rarer organism than the pneumococcus, and proportionately less important.

Diplococcus Intracellularis.—Each is 1 μ in

diameter; they are arranged in pairs, with no capsule, and occur both inside and outside the pus cells. They do not retain the stain by Gram's method, and are difficult to distinguish, by the microscope alone, from gonococci.

Importance.—They give rise to posterior basal and to epidemic cerebro-spinal meningitis, in which the prognosis is less hopeless than in the tuberculous or suppurative forms. They are present in the pus beneath the pia mater, and may sometimes be found in the cerebro-spinal fluid obtained by lumbar puncture.

Bacillus Coli Communis.—Each is a short rod, with blunt, rounded ends, measuring 3 μ by 0·5 μ. In the fresh state it is motile by means of flagella, which require special and somewhat difficult methods of staining. It does not form spores.

It does not retain the stain by Gram's method.

Importance.—Occurring abundantly in the intestinal tract, it is there harmless. Escaping to other tissues, it may become very virulent, and is found in many cases of appendicular abscess, general suppurative peritonitis, vulvitis, cystitis.

Fig. 52.—Bacillus Typhosus.

Bacillus Typhosus.—A rod-shaped organism, 3 μ by 0·5 μ, with rounded ends (Fig. 52), and flagella which are difficult to stain. It is motile when alive, does not form spores, loses the stain by Gram's method, and is quite indistinguishable by the microscope alone from *Bacillus coli communis*.

Importance.—It is very little use looking for the organism microscopically, for without pro-

longed cultural processes it is impossible to distinguish it from the *Bacillus coli communis*. It occurs in the stools, mesenteric glands, spleen, subcutaneous abscesses in typhoid fever, and sometimes in the urine during convalescence.

DIPHTHERIA BACILLI.—Slender rods about 3 μ by 0·4 μ, but varying considerably. They are non-motile, and do not form spores, though they often show beads of lighter and darker staining which resemble spores (Fig. 53). They retain the stain by Gram's method. It is very important in a doubtful case of sore throat to examine for the bacilli. Depress the patient's tongue with a spatula whilst he breathes through his mouth; collect a little of the suspected matter from the tonsil upon a pledget of cotton-wool; smear the latter over a clean cover-slip; fix the film by heat, and stain by Gram's method. The following is a special method for staining them:

FIG. 53.—Pus Cell and Diphtheria Bacilli.

Neisser's Stain for Diphtheria Bacilli.

Methylene blue (Grübler's)	1 gramme
Rectified spirit	20 c.c.
Distilled water	950 ,,
Glacial acetic acid	50 ,,

Contrast Stain.

Vesuvin	2 grammes
Distilled water	1,000 c.c.

Boil; allow to stand; filter; and store for use.

Immerse the film in Neisser's stain for four seconds; rinse rapidly in distilled water; immerse in the con-

trast stain for four seconds; rinse in distilled water; blot; dry in the air; mount in Canada balsam.

Diphtheria bacilli will be stained brown with an occasional bright blue granule in their interior. Their presence may thus be established in a few minutes, and under conditions when there may be no facilities for employing cultural methods.

Importance.—Many cases of diphtheria need no such examination to assist the diagnosis. Many others cannot be diagnosed from septic sore throat or follicular tonsillitis without it. On the other hand, diphtheria bacilli sometimes occur in the throats of people who are seemingly healthy, so that, without clinical signs as well, the mere presence of the organisms does not constitute diphtheria.

ANTHRAX BACILLUS.—Each is a characteristic and comparatively large organism, 7 μ long and 1·2 μ broad, non-motile, spore-forming, and many may be joined end to end in a thread-like chain (Fig. 54).

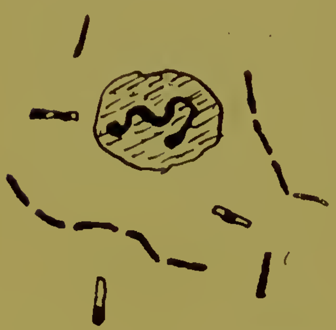

FIG. 54.—Anthrax Bacilli and Pus Cell.

It retains the stain by Gram's method.

Importance.—Though rare in many places, it occurs not infrequently in those who work with raw foreign hides or wool—for example, in the tanyards of Bermondsey. The common lesion is a local 'malignant pustule' on the face or hand. Early detection of the organisms is all-important.

Whilst they are local to the pustule, excision of the latter saves the patient; after infection of the blood-stream, the disease is beyond control.

TETANUS BACILLUS.—Each is a slender rod, 5 μ long by 0·4 μ broad. They do not form chains as a rule, and, when fresh, are very slightly motile. Their chief characteristic is the formation of a large bulging spore at one end, which gives them a 'drum-stick' shape (Fig. 55).

FIG. 55.—Tetanus Bacilli.

The organism stains by Gram's method.

Importance.—It is by the clinical signs that the disease is diagnosed. The discovery of the organism has its chief value in determining where the inoculation has been, and, consequently, the part requiring surgical attention. The bacillus is anaerobic, and occurs locally in some septic wound; along with it numbers of other organisms are usually present.

ACTINOMYCOSIS.—This is rare in man, but affects four different parts of the body; least rarely the lower jaw, and very rarely the cæcum, the liver, or the lung. To the naked eye the pus contains minute granules, never bigger than a small pin's head, usually semi-translucent and of a greenish-gray. Quite rarely their colour is an orange yellow. Under the microscope (Fig. 56) each granule is seen to have three parts:

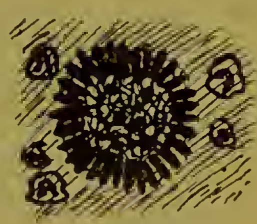

FIG. 56.—Actinomyces and Pus Cells.

1. A central *mycelium* of interlacing threads 0·5 μ in diameter.

2. *Cocci*, or small spore-like bodies, intermingled with the mycelium.

3. *Clubs*, the swollen, pear-shaped ends of filaments which project radially outwards from the central felt-work, and give the 'rayed' form to the mass.

The mycelium and cocci stain both with the ordinary aniline dyes and by Gram's method; the clubs stain with the former, but not by Gram's process.

Importance.—Notwithstanding the rarity of the affection, its diagnosis from other forms of suppuration is all-important, because potassium iodide may cure it without need for operative measures.

GLANDERS.—The *Bacillus mallei* is a minute rod, 3 μ by 0·4 μ, with a tendency to form short chains. It is non-motile, does not form spores, and does not stain by Gram's method.

Importance.—Not very great, because of the rarity of the disease in man. It does occur sometimes in those who have to do with horses. The lesions are in the skin or in the lung, and resemble those of tubercle; the diagnosis can only be made certain by discovering the organism in the caseous pus.

FIG. 57.—Cholera Vibrio.

CHOLERA VIBRIO.—Found in the stools rather than in pus. It possesses a terminal flagellum, and is motile when alive. In stained films each is a bacillus curved like a comma (Fig. 57)—broader at one end than the other. It is

very small, 1·5 μ by 0·5 μ, and there is a tendency for two to join end to end, with their curves opposite ways, like the letter S.

It loses the stain by Gram's method.

Importance.—It is found in the rice-water stools, and is of diagnostic value in sporadic cases of cholera.

PLAGUE BACILLI.—Small oval rods, shorter than the typhoid bacillus, but of the same thickness. The ends are rounded, and the central part remains unstained, giving a characteristic 'pole-staining.' They do not form spores, are non-motile, and occur singly as a rule, though they may form chains.

FIG. 58.—Plague Bacilli.

They are decolourized by Gram's method.

Importance.—In a doubtful case of plague these organisms should be looked for in the affected glands or buboes, or, in pulmonary cases, in the sputum.

For TUBERCLE BACILLI, see p. 87. Typical tuberculous pus shows flakes of cheesy material suspended in thin yellow fluid; on microscopical examination it is frequently impossible to find any bacilli at all.

For the AMŒBA OF DYSENTERY, see p. 123. This gives rise to hepatic abscess, the pus from which has a characteristic chocolate and milk colour. When obtained from the interior of the abscess, the pus is often sterile; but if scrapings from the abscess wall be taken, the amœba is usually demonstrable.

CHAPTER V.

EXAMINATION OF THE GASTRIC CONTENTS.

A. CHEMICAL EXAMINATION.

The *apparatus* required is:

One glass funnel 6 inches in diameter.
One glass funnel 2 inches in diameter.
Two glass beakers of 300 c.c. capacity.
One glass flask of 1,000 c.c. capacity.
One burette for decinormal soda solution.
One pipette, 100 c.c.
One pipette, 10 c.c.
Four small evaporating basins of 50 c.c. capacity.
One small flat porcelain slab.
Test-tubes.
Fine muslin for filtering.
Filter-papers for the smaller funnel.
Litmus-papers, blue and red.
Bunsen burner, or spirit lamp.
Iron tripod.

Reagent bottles, conveniently 2 or 4 ounce capacity; one big bottle for standard soda solution; one flat

saucepan, about 6 inches in diameter and about 2 inches deep, for use as a water-bath.

The following *reagents* are necessary:

Gunsberg's Reagent.—A test for free hydrochloric acid:

Phloroglucin	..	..	..	2 grammes
Vanillin ..	..	..	..	1 gramme
Rectified spirit	..	..	..	100 c.c.

Keep the bottle stoppered and not exposed to bright light. The solution need not be freshly prepared; but it loses its delicacy as a test in time, so that fresh should occasionally be made.

Congo-red Test Papers.—A test for free acid, organic and inorganic:

Congo red ..	..	..	..	0·1 gramme
Water ..	..	..	..	100 c.c.

Into the solution dip filter-paper, allow the latter to dry, cut it into strips, and store ready for use.

Uffelmann's Reagent.—A test for lactic acid:

Carbolic acid	..	..	..	3 grammes
Distilled water	..	..	..	100 c.c.

To a little of this solution, about 1 inch in a test-tube, add 1 or 2 drops of liquor ferri perchlor. (B.P.); an amethyst blue colour results. This is the complete reagent, but it does not keep, so that the ferric chloride and carbolic acid solutions should be kept in separate bottles, and mixed when required.

Decinormal Soda Solutions for the Estimation of Acidity.—A decinormal solution is such as contains one-tenth part of the molecular weight, in grammes, of a univalent substance, dissolved up to 1,000 c.c., usually in water. The cheapest method in the case of soda is to use

sticks of caustic soda, but it is difficult to weigh it accurately, because it absorbs water from the air. The molecular weight of NaHO is 23 + 1 + 16 = 40, so that 4 grammes of solid caustic soda, dissolved up to 1,000 c.c. in water, makes a decinormal solution.

An equivalent solution of sodium carbonate would contain 5·3 grammes. The objection is that this salt is seldom pure, but a varying mixture of $NaHCO_3$ and Na_2CO_3.

If caustic soda be used, the best plan is to make the solution as accurately as possible; then to titrate it with a known solution of sulphuric acid, and thus determine its actual strength. Provided the strength of the soda solution be accurately known, it matters little whether it be absolutely decinormal or not.

The following is an accurate but more expensive method: Weigh a small test-tube, dried and corked; with a clean knife cut the outside crust from one or more pieces of pure sodium; transfer the bright metal to the test-tube; cork, and weigh again. Deduct the weight of the test-tube, and cork to find that of the sodium; transfer the latter to a clean flask containing about 200 c.c. of absolute alcohol. It dissolves with evolution of heat, but with less violence than in water; it forms sodium ethylate, which can then be diluted with distilled water to the calculated volume and kept for use. Every 2·3 grammes of sodium must be diluted up to 1,000 c.c. to make a decinormal solution, of which 1 c.c. corresponds to 0·0023 gramme of sodium, or to 0·004 gramme of Natto, or to 0·00365 gramme of HCl.

Phenolphthalein Solution.—The indicator in estimating acidity :

Phenolphthalein	0·2 gramme
Rectified spirit	60 c.c.
Distilled water to	100 ,,

The solution is colourless in the presence of even weak acids, and is turned rose red by alkali.

Discs of hard-boiled White of Egg for Testing Pepsin.—Boil an egg for twenty minutes; let it get cold; remove the shell; peel the white from the yolk, cut the former into strips, and punch out discs with a cork borer. Put the discs into glycerine in a small bottle ready for use.

The following reagents are not necessary, but may be employed as additional tests for free hydrochloric acid :

Boas's Reagent.

Resorcin	5 grammes
White sugar	3 ,,
Rectified spirit	100 c.c.

Tropæolin OO.

Methyl orange	0·2 gramme
Rectified spirit	25 c.c.
Distilled water to	100 ,,

Methyl Violet, or Pyoktanin.

Methyl violet	0·05 gramme
Distilled water	100 c.c.

METHOD OF PROCEDURE.—First, *strain the vomit* through a piece of fine muslin, using the large funnel and flask. Do not use filter-papers, as these alter the acidity of the filtrate by allowing certain of the acid substances to pass through with greater readiness than others; they also delay filtration.

Next, *test the filtrate with blue litmus-paper.* The vomit may be alkaline. On the other hand, if blue litmus be turned red, the 'acidity' may be due to one or more of four causes:

(*a*) Acid salts (acid sodium phosphate).

(*b*) 'Combined' hydrochloric acid; that is, molecularly combined with the proteid. In normal digestion after a proteid meal, the first part of the hydrochloric acid secreted combines loosely with the proteid molecules; during this time salivary digestion of carbohydrates continues, lactic acid is usually found, and it is not until about an hour later that enough hydrochloric acid has been secreted to leave a surplus 'free.' From this time onwards more 'free' hydrochloric acid is present and less lactic, until the hydrochloric acid reaches 0·2 per cent. and lactic acid is absent.

(*c*) Organic acids, chiefly lactic, with traces of acetic and butyric.

(*d*) 'Free' hydrochloric acid.

To determine the Causal Factors of the Acidity.

Test with a Congo-red Paper.—If the colour be not altered, neither organic acid nor free hydrochloric acid is present; the acidity is due to acid salts and combined hydrochloric acid. If the paper be turned blue, both free hydrochloric and lactic acids may be present. If the red be changed to a dirty brown, lactic acid is probably present without free hydrochloric.

To confirm the presence of free hydrochloric acid, *test with Gunsberg's reagent* as follows: Boil some

water in the saucepan, as a water-bath. Put 3 drops of Gunsberg's fluid and 3 drops of the vomit filtrate into a small evaporating dish; float the latter on the boiling water. Free hydrochloric acid is indicated by a beautiful rose-red colour appearing, first at the drying margins, and later all over the evaporated residue. The test is easy; but is liable to fail if the vomit be not strained, if more than a few drops of either vomit or reagent be used, or if the water-bath be dispensed with and direct heat employed.

Gunsberg's test gives a similar rose-red colour with any free inorganic acid, such as nitric or sulphuric, also with *strong* solutions of organic acids. There is never sufficient organic acid in the vomit to do this, nor are nitric or sulphuric acids present; hence Gunsberg's is one of the best for free hydrochloric acid in the stomach contents.

Next use *Uffelmann's test for lactic acid.* Fill 1 inch of a test-tube with the reagent, add strained vomit drop by drop until the amethyst blue colour is discharged. Free hydrochloric acid makes the solution colourless, lactic acid a pale canary yellow. It is the *solution* that is yellow. Frequently, on adding acid vomit, a yellowish cloud of phosphates is formed; an alkaline vomit gives a pale orange colour due to a precipitate; in all cases *filter* the mixture. If the *filtrate* is clear pale yellow, lactic acid is present; if a faint yellow, there is a trace; if colourless, none.

The additional tests mentioned above are performed as follows:

Boas's reagent is used exactly as Gunsberg's. A similar, but fainter, rose red indicates free hydro-

chloric acid. Longer heating is necessary, the rose colour sometimes not appearing for ten minutes after evaporation is complete. Allowing for this, Boas's test is almost as satisfactory as Gunsberg's, is cheaper, and the solution keeps good longer.

Tropæolin Test.—Place a drop of the solution upon a porcelain slab, spread it out in a thin layer, and dry it at 40° C. To do this place the slab close to the foot of the Bunsen burner. When dry, put a single drop of the strained vomit upon the centre of the yellow stain, and continue warming at the foot of the burner as before. A beautiful violet colour appears if free hydrochloric acid be present. A similar violet is given by *strong* lactic acid, but not with the amount present in a vomit. The ease of this test strongly recommends itself in private practice.

The Methyl Violet Test is also very simple. Put 1 drop of methyl violet solution upon a white dish close to 1 drop of the strained vomit. A change from violet to bright greenish-blue at their point of coalescence is regarded by many as proof that free hydrochloric acid is present. There are, however, conditions in which sufficient lactic acid may be present to produce a similar change.

Summary:

With blue litmus-paper, a red reaction indicates one or more of the following: Acid salts, combined hydrochloric acid, lactic acid, free hydrochloric acid.

With Congo red, a blue reaction indicates lactic acid or free hydrochloric acid. A brown reaction indicates lactic acid, probably no free hydro-

chloric. With acid salts or combined hydrochloric acid, the red does not change.

With Gunsberg's reagent, a rose-red colour indicates free hydrochloric acid.

With Uffelmann's reagent, a yellow colour of the *filtrate* indicates lactic acid.

Boas's reagent (rose red) Tropæolin OO (violet) Methyl violet (to greenish-blue)	May be used as alternatives to Gunsberg's test.

Importance of the Tests.—The absence of free hydrochloric acid from vomit within an hour of proteid food is physiological. Its absence later indicates deficient acid secretion, resulting in interference with peptic activity and a liability to the growth of organisms. Knowledge of the deficiency is an important indication for treatment, the result of which may be gauged by subsequent testings.

In gastric carcinoma free hydrochloric acid is often absent, a fact to which diagnostic value has been attached. But it may be absent in many other conditions; for example, in fevers, chronic heart disease, Bright's disease, and in almost any debilitating malady.

Presence of lactic acid is not pathological for the first hour after food; longer than this its presence in quantity indicates undue fermentation, which requires treatment.

To Estimate the Acidity.

The Total Acidity.—Fill the burette with decinormal soda solution. Pipette 100 c.c. of strained vomit into

a beaker. Add 10 drops of phenolphthalein solution. Place the beaker upon a white slab under the burette. Add the soda, a few drops at a time, until a definite pink colour appears and persists. It is well to have a second beaker containing colourless acid solution for comparison. The volume of soda solution added gives the amount of caustic soda needed to neutralize the acids in the 100 c.c. of vomit.

The Acidity due to Free Hydrochloric Acid.—Most methods are too complicated for practical use. The following is simple, and gives approximate results: Prepare a series of small evaporating basins, and into each put 2 drops of Gunsberg's reagent. Pipette 100 c.c. of strained vomit into a beaker. Add decinormal caustic soda from the burette; after the addition of 5 c.c., put 2 drops of the beaker contents into the first evaporating basin, and dry the latter on the water-bath. Add 5 c.c. more soda to the beaker, put 2 drops of the beaker contents into the second basin, and dry it on the water-bath. Repeat this operation with successive evaporating basins after each addition of 5 c.c. decinormal soda. Suppose a deep rose red results in the first basin, a less deep with the second, a faint with the third, and none at all with the fourth, it follows that between 15 c.c. and 20 c.c. of decinormal soda are needed to neutralize the free hydrochloric acid in 100 c.c. of vomit. Now pipette 100 c.c. of strained vomit into a fresh beaker; prepare the basins with Gunsberg's reagent as before; add 16 c.c. of soda to the beaker, and take 2 drops from the latter for the first basin; and similarly 2 drops for successive basins after 17 c.c., 18 c.c., and 19 c.c.,

of soda have been added respectively. If the rose-red reaction be faint in the first basin, fainter in the second, just perceptible in the third, and absent in the fourth, it follows that between 18 c.c. and 19 c.c., or 18·5 c.c. of decinormal soda solution just neutralize the free hydrochloric acid in 100 c.c. of vomit.

If the total acidity be already estimated, the difference between the two gives the acidity due to lactic acid, combined hydrochloric acid, and acid salts.

Importance of Estimation.—It is unusual in health to find more than 0·2 per cent. of free hydrochloric acid. More indicates hyperacidity, and needs correction. This may be accomplished by prescribing more proteid food to combine with the hydrochloric acid, or by ordering alkalies to neutralize the acid at that period after a meal when it is present in excess.

When doubt exists in diagnosis between gastric carcinoma and a simple ulcer, hyperacidity favours the latter.

To Test for Pepsin.

Into each of four test-tubes put 5 c.c. of strained vomit and 1 disc of egg-white. To the second add 2 grains of dried pepsin, to the third 2 drops of dilute hydrochloric acid (B.P.), to the fourth 2 drops of dilute hydrochloric acid and 2 grains of pepsin. Warm the saucepan water-bath to 40° C., stand all the test-tubes in it, and leave them at this temperature for two hours. The egg in the fourth tube will have swollen up and been partly dissolved. If it be dissolving in the third, and not in the first, acid is deficient in the vomit, but

pepsin is present. If it be dissolving in the second, and not in the first, pepsin is deficient. If it dissolve in the fourth, but not in the first three, both pepsin and acid are deficient.

Importance.—The absence of pepsin has no certain diagnostic value, but indicates the need of prescribing pepsin to aid proteid digestion.

To Test for Rennin.

Neutralize 10 c.c. of strained vomit by adding 2 drops of phenolphthalein as an indicator, and dilute caustic soda until a faint pink colour appears. Add 10 c.c. of fresh cow's milk previously boiled and allowed to cool. If rennin be present, the caseinogen of the milk will be precipitated in flakes.

Importance.—If rennin be absent, the caseinogen of milk forms dense curdled masses in the stomach. In cases of vomiting upon milk diet, test for rennin; in its absence, the precipitate of casein in the stomach may be rendered finer by mixing the milk with barley or lime water, or by giving rennet in the form of curds and whey.

B. MICROSCOPICAL EXAMINATION.

Mix a little of the deposit from the muslin filter with a drop of salt solution upon a slide, put on a cover-slip, and examine it with a low power.

Particles of food will be seen. *Fat droplets*, highly refractile structureless globules, large and small; staining black with a 1 per cent. solution of osmic acid. *Starch grains*, round or oval, concentrically

striated; staining blue with dilute iodine. *Elastic fibres*, double contoured, sometimes branched and twisted spirally at their free ends; staining deep purple, almost black, with magenta. *Muscle fibres*, transversely striated, unmistakable.

Importance.—Fat droplets are of no importance. Starch grains, elastic fibres, muscle fibres, may be found when no food containing any of these has been eaten the same day. They then indicate delayed emptying of the stomach, perhaps from poor motility, perhaps from pyloric obstruction.

VEGETABLE PARASITES.—For these the $\frac{1}{6}$ inch power is needed. Two sorts may be looked for:

Sarcinæ ventriculi, or 'packet cocci,' each resembling a bale of wool tightly corded, so as to bulge between the cords. There may be four cocci in each packet,

FIG. 59.
A, sarcinæ ventriculi; B, yeast fungi.

or any multiple of four (Fig. 59). Each coccus is about the size of a leucocyte, has a yellow tinge, and with iodine stains mahogany-brown.

Yeast fungi, or torulæ, round or oval cells in branching chains or clusters (Fig. 59).

Importance.—Neither is present in health. They indicate chronic delayed emptying of the stomach; common in dilated stomach, whatever the cause.

Test Meals.

Carbohydrate stimulates the flow of gastric juice less than does proteid, but leads to an earlier appearance of free hydrochloric acid. The more proteid given, the less early is free hydrochloric acid to be expected (see p. 110). Before interpreting the results of an examination, it is essential to know what food has been taken, and at what interval before the stomach contents were obtained. In such cases as require particular investigation, therefore, it is usual to give a 'test meal,' and to recover it by the stomach-pump. The test meals recommended are:

Ewald's Breakfast (Eight O'clock).

White bread	70 grammes
Weak tea	300 c.c.

Empty the stomach in one hour.

Klemperer's Breakfast (Eight O'clock).

White bread	70 grammes
Milk	500 c.c.

Empty the stomach in two hours.

Germain See's Lunch (Eleven O'clock).

White bread	150 grammes
Minced meat	80 ,,
Cold water	300 c.c.

Empty the stomach in two hours.

Riegel's Dinner (Two O'clock).

Soup	100 c.c.
Beef-steak	60 grammes
White bread	50 ,,

Empty the stomach in five hours.

In practice, Ewald's breakfast is the most convenient.

CHAPTER VI.

EXAMINATION OF THE FÆCES.

A. GENERAL EXAMINATION.

The fæces are extremely variable in amount, form, colour, and odour, but the following types are never healthy:

The 'Pea-soup' Stool.—Copious, unformed, dirty yellow, very ill-smelling. Chiefly seen in typhoid fever, but by no means always present in this disease.

The 'Rice-water' Stool.—Colourless, odourless, alkaline, watery, with numbers of small white flocculi of epithelium and mucus floating in it. It is seen in cholera, but very similar stools may occur in severe arsenical poisoning and in the very severe diarrhœa that occasionally results from cathartic drugs.

The Blood and Mucus Stool.—Small in amount, without fæcal colour, often odourless, consisting of slimy mucus mixed with bright-red blood. It occurs in several conditions, notably intussusception, acute colitis, ulcerative colitis, dysentery.

The Clay-coloured Stool.—May be well or ill formed; usually offensive, but not always so. The colour resembles that of putty, and is due to absence of bile pigments. It indicates obstruction of the large

bile-ducts, as by catarrh, gall-stone, or growth. It is, therefore, associated with jaundice ; but jaundice may occur without clay-coloured stools when the large bile-ducts are free, as in cirrhosis of the liver.

The Fatty, Clay-coloured Stool.—When bile is absent fat digestion is imperfect, but when neither bile nor pancreatic juice reach the bowel it almost ceases. A copious iridescent scum of fat upon a clay-coloured motion suggests occlusion of both bile and pancreatic ducts, such as results from carcinoma of the head of the pancreas.

The Infantile Zymotic Enteritis Stool.—Very scanty, drying upon the diaper as a grass-green stain of a most offensive odour; occasionally streaked with blood.

The Purulent Stool.—Obvious pus occurs sometimes, coating the motion, or coming from the bowel as a separate evacuation. It may occur in dysentery or ulcerative colitis, but more often follows rupture of an appendicular or other abscess into the colon or rectum.

The Membranous Colitis Stool.—Consists of mucin in the form of white gelatinous casts of the interior of the bowel. They have every variety of length, from an inch up to some feet ; are often narrow and riband-like, suggesting tape-worms ; but when floated in water they usually show a central lumen, and they are not segmented. They are diagnostic of membranous colitis, a most troublesome, if somewhat rare, disorder, occurring more often in women than in men.

The Stool with Blood in it.—The character of

blood in a motion varies with the site of the bleeding-point. Bright streaks of blood upon the outside of a stool indicate hæmorrhage near the rectum; *e.g.*, from piles or sigmoid or rectal carcinoma or other stricture. Bright blood mixed with the fæces comes from higher up—*e.g.*, from tuberculous or other ulceration of the ileum or cæcum. Blood and mucus occur with intussusception or inflammation or ulceration of the colon. When bleeding is high up—*e.g.*, from a gastric or duodenal ulcer—or when blood has been swallowed and passed on, the motions are black and tarry, a condition called melæna.

Fallacies of Melæna.—Black motions may also be due to:

1. Certain drugs.

Iron. Manganese. Bismuth.	Converted by the sulphuretted hydrogen in the bowel into sulphides.

2. Certain foods.
   Bilberries.
   Charcoal biscuits.

To confirm the presence of blood, a little of the motion may be smeared upon a piece of lint, a drop of tincture of guaiacum placed by the side of it, and ozonic ether poured over both. If blood be present, a bright blue colour appears. The guaiacum reaction, however, has many fallacies (p. 20); and a more conclusive test is with the spectroscope. Shake up a little of the fæces with water in a test-tube, allow the solid particles to settle, and examine the supernatant fluid for the absorption bands of hæmoglobin (p. 21) or of hæmatin.

B. SPECIAL EXAMINATIONS.

GALL-STONES.—Mix the fæces with a bulk of water in a porringer, and stir well with a stick. Pour on to a fine meshed sieve. If necessary, continue to pour water on to the sieve, triturating the fæcal residue with the stick at the same time. The gall-stone will become visible after the softer fæcal matter has passed through. It should be picked out and tested as follows:

1. *For Cholesterin.*—Dissolve a portion of the stone in chloroform; add sulphuric acid. If cholesterin be present the solution assumes a blood-red colour, going on to purple red.

Or, extract a part of the stone with ether; evaporate the solution; dissolve the residue in absolute alcohol, and allow to crystallize. The characteristic crystals of cholesterin (Fig. 60) may be detected under the low power.

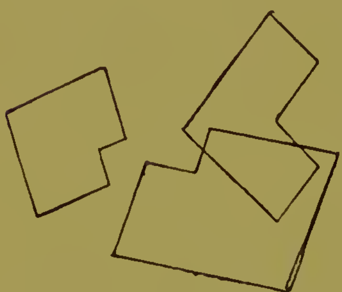

FIG. 60.—Cholesterin Crystals.

2. *For Bile Pigments.*—Dissolve a portion of the stone in weak hydrochloric acid. Apply Gmelin's test (p. 31) to the solution.

Importance.—Diagnosis is confirmed in obscure cases of colic. Should the stone be facetted, other gall-stones are, or have been, present.

TYPHOID SLOUGHS.—These may be looked for on a sieve in the same way as gall-stones.

Importance.—Less now than formerly. Previous

to the discovery of the Widal test, the diagnosis of typhoid fever often depended on finding sloughs from Peyer's patches in the stools.

UNDIGESTED CURDS OF MILK. — These may be readily recognised.

Importance.—In many cases—*e.g.*, typhoid fever —milk diet may cause diarrhœa. Before ordering astringents, examine the fæces. Should curds be present, modify the milk by adding lime or barley water to it, or by peptonizing it, and the diarrhœa will often cease.

BACTERIA.—These swarm in fæces, and are for the most part unimportant. To examine them, pick out a very small portion of the excretion with forceps, make a film upon an *albuminized* cover-slip; fix in the flame, and stain by the methods described on p. 95. The organisms of importance are:

The Cholera Vibrio (p. 104), obtainable from the rice-water stools.

The Amœbæ of Dysentery.—These can only be found in stools less than twelve hours old; later they disintegrate. They are best obtained from the smaller pellets of mucus; each is a rounded or irregular protoplasmic mass about 30 μ in diameter, finely granular, refractile, with several vacuoles, and ingested red corpuscles or bacteria (Fig. 61). In warm 0·6 per cent. salt solution they may be watched sending out blunt pseudopodia, and gradually crawling along the slide. In the fresh state they show no nucleus, but stained specimens show one about the size of a red corpuscle. A film may be made upon an albuminized cover-slip; it cannot be fixed by heat because the amœbæ dis-

integrate, but should be immersed for five minutes in the following solution :

Gulland's Fixing Solution.

Absolute alcohol	25	c.c.
Pure ether	25	,,
Solution of corrosive sublimate in absolute alcohol (strength, 2 grammes in 10 c.c.) ..	0·5	,,

and then stained with carbol methylene blue or with carbol fuchsine (p. 95).

Importance. — There are probably several

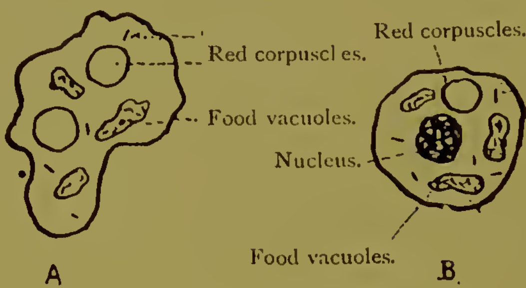

FIG. 61.—Amœbæ of Dysentery.
A, fresh ; B, fixed and stained.

varieties of dysentery, due to different organisms. Hepatic abscess is more liable to follow amœbic dysentery than other forms.

THE TYPHOID BACILLUS, unfortunately, is so similar to the *Bacillus coli communis* (p. 100) that it cannot be discriminated by ordinary methods.

PARASITES.

These are of two classes : I. Cestodes, or tape-worms. II. Nematodes, or thread-worms.

I. CESTODES. — These have two life cycles: a

sexual, the hermaphrodite tape-worm, in one host; and an asexual, cystic, in another. There are three whose tape-worm stage occurs in the human small intestine—namely:

Tænia Solium.—Intermediate host, the pig; length, 10 feet; head (Fig. 62) has four round sucking discs and a single row of hooklets, about twenty-six in number.

Fig. 62.—Head of Tænia Solium.

Tænia Mediocanellata.—Intermediate host, the ox; length, 15 feet; head (Fig. 63) has four round sucking-discs, and no hooklets.

Bothriocephalus Latus.—Intermediate host, fish, such as the pike; length, 20 feet; head (Fig. 64) has two longitudinal sucking-discs, and no hooklets.

Fig. 63.—Head of Tænia Mediocanellata.

These worms are composed of hundreds of opaque white segments joined end to end. Each segment is a complete hermaphrodite organism, containing an ovary, a testis, and a water-channel system. At the upper end of the worm is a narrow neck, and a head not much larger than that of an ordinary pin. The hinder segments, becoming detached from time to time, appear in the fæces. After the administration of anthelmintics, diligent search must be made for the head, for there is no certainty of the eradication of the worm until the head itself has been passed. It is by the characteristics of the head that the variety of tape-worm is diagnosed.

(*Note.* — The head of *Tænia echinococcus* has four round sucking-discs like that of *Tænia solium* but forty hooklets in two rows instead of twenty-six hooklets in one. The worm, $\frac{1}{4}$ inch long, consists of a head, a neck, an immature and a mature segment; it inhabits the intestine of the dog, and it is only the cystic stage which affects man, causing hydatids.)

Fig. 64.—Head of Bothriocephalus Latus.

II. Nematodes.—These have the sexes distinct, and are not known to have an asexual generation in any intermediate host.

Ascaris Lumbricoides.--This is 6 or 8 inches long; is not unlike an earth-worm, but is distinguished by the following points:

1. There is no imbrication of segments, so that the finger can be drawn along it either way without catching.

2. The mouth is at the extremity instead of under a blunt prostoma, as in the earth-worm.

3. It tapers towards each end.

4. It has no cingulum or fleshy band, such as surrounds the earth-worm at the junction of the anterior and middle thirds.

It inhabits both small and large intestine.

Importance.—It is often the cause of obscure nerve disorders, convulsions, or night terrors in children. Sometimes persons produce an earth-worm, and state that their child has passed it.

Oxyuris Vermicularis.—This is $\frac{1}{3}$ inch long, blunt at the head end, tapering to a pointed tail (Fig. 65), and appearing in the fæces like white, slowly-moving short

threads. It inhabits the large intestine, and is very common in children. Enormous numbers may occur in the same patient.

Importance.—Besides the common gastro-intestinal and nervous symptoms, these worms may give rise to vulvitis in girls; the condition may rouse a suspicion of gonorrhœa, or lead to an accusation of rape.

FIG. 65.—Oxyuris Vermicularis.

Ankylostoma Duodenale.—This inhabits the duodenum, and does not occur spontaneously in the fæces. Should ankylostomiasis be suggested by severe and obscure anæmia, thymol should be administered, and the fæces then examined for the worms. Each is $\frac{1}{4}$ inch long, grayish-white in colour, cylindrical; the posterior end is broad, and in the male is provided with an umbrella-

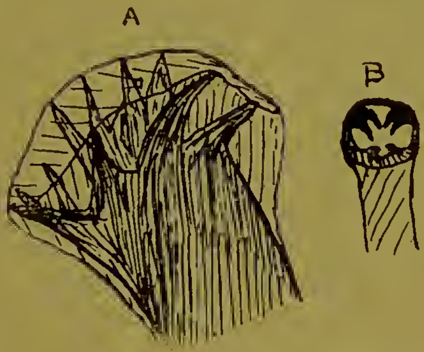

FIG. 66.—Ankylostoma Duodenale.
A, tail of male; B, head.

like expansion with eleven ribs (Fig. 66). The worm tapers forwards to a very narrow neck, which ends in a bulging mouth, the margin of which is furnished with four characteristic claw-like hooks and two blunt teeth, which enable the parasite to adhere firmly to the duodenal wall.

Importance.—The symptoms of ankylostomiasis are so similar to those of pernicious anæmia that cases are easily overlooked. Eosinophilia may suggest the diagnosis, which is confirmed by giving thymol and finding the parasite in the fæces.

Trichocephalus Dispar.—This is about $1\frac{1}{2}$ inches long, and consists of an opaque white body which is often wound up like a watch-spring, and a long thread-like anterior extremity (Fig. 67), which is embedded in the wall of the bowel. It inhabits the cæcum.

FIG. 67.—Trichocephalus Dispar.

Importance.—Though not uncommon, it has no importance; it causes no symptoms.

(*Note.*—Both Cestodes and Nematodes lay enormous numbers of eggs, which may be recognised under the microscope by their oval outline, clear capsule, and cellular interior containing the coiled-up embryo. The ova of different species are too similar to be distinguished without considerable practice.)

CHAPTER VII.

MICROSCOPICAL EXAMINATION IN AFFECTIONS OF THE SKIN.

RINGWORM.—Though more than two varieties have been described, it is sufficient for practical purposes to make the following two classes:

1. *Tinea tonsurans*, ringworm of the scalp, due to a vegetable parasite, which forms a mycelium inside the hairs, produces small spores, and is called the *Trichophyton microsporon endothrix.*

2. *Tinea circinata*, ringworm of the body, due to a vegetable parasite, which forms a mycelium outside the hairs, produces large spores, and is called the *Trichophyton megalosporon ectothrix.*

There is a third parasite described, with large spores and its mycelium inside the hair—the *Trichophyton megalosporon endothrix.* Apparently this is much rarer than the other two.

The essential things to look for are the mycelium and the spores. With forceps pick out one of the stubbly hairs from the suspected patch, and proceed by one or other of the following methods:

(*a*) Without staining. Immerse the hair in ether for fifteen minutes, or for twenty-four hours if there be

time, in order to remove the grease. Transfer to liquor potassæ for fifteen minutes. Wash in water, and place in a drop of glycerine upon a slide; apply a cover-slip; press it down; blot up the excess of glycerine; ring the specimen with Canada balsam, and examine under the $\frac{2}{3}$ inch objective. The mycelium within or around the hair will be readily seen.

(*b*) Stained specimen. Immerse in ether to remove the fat as before. Stain the hair by Gram's method (p. 96). Mount in glycerine; or dehydrate with absolute alcohol, clear with xylol, and mount in Canada balsam. Examine under $\frac{1}{6}$ inch power. The spores will be deep violet, as they retain the stain by Gram's method.

Whether the mycelium be within the hair or upon the outside, the spores are for the most part on the surface. In the case of *Tinea tonsurans*, the small spores form a closely-packed casing to the hair, resembling a mosaic (Fig. 68). In *Tinea circinata*, on the other hand, the large spores are less densely packed, and form rows, which have been compared to rosaries (Fig. 69).

FIG. 68.—Spores in Tinea Tonsurans.

FIG. 69.—Spores in Tinea Circinata.

Importance.—It is much easier to cure the *Tinea circinata* than the *Tinea tonsurans*, because oint-

ments more readily reach the mycelium which lies outside the hair than that which lies within.

Tinea Versicolor.—With the back of a scalpel scrape the surface of one of the brown patches; transfer the fine scrapings to a drop of liquor potassæ on a slide; apply a cover-slip, and examine under $\frac{1}{6}$ inch objective. Amongst dirty-looking clumps of epithelial débris will be seen the *Microsporon furfur*, interlacing jointed threads of colourless mycelium with bunches of spores between them (Fig. 70).

Fig 70.—Microsporon Furfur.

Importance. — *Tinea versicolor* may be mistaken for a true pigmentation of the skin, such as that of Addison's disease. By demonstration of the fungus error is avoided.

Favus.—Pick out a hair and as small a portion of the surrounding crust as possible. Transfer it to a drop of liquor potassæ on a slide, apply a cover-glass, press it down, and examine with a $\frac{2}{3}$ inch objective. A fungus, the *Achorion schœnleinii*, will be seen as a dense branching mycelium, completely infiltrating the hair, and forming a dense coating of large spores upon its surface. It closely resembles a trichophyton.

Importance.—The affection is rare in England. A typical case is easy of recognition; but if the primary affection be obscured by impetigo, diagnosis without the microscope may be difficult.

SCABIES.—The parasite is called *Sarcoptes hominis*, or *Acarus scabiei*, and is related to the spiders. There are two sexes. The male lives upon the surface of the skin, is the smaller, and is very difficult to find. The female, when impregnated, penetrates the stratum corneum, and burrows along in the rete Malpighii, depositing eggs as she goes. Her point of entry is marked by a vesicle, and her path by a typical black 'run.' To obtain the parasite, lay a pin flat upon the surface of the skin, and push it with a rotatory movement into the epidermis at the end of the burrow away from the vesicle, taking care not to draw blood. The acarus appears as a small pearly object at the end of the pin. Under the microscope it is oval, with eight legs, two in front and two behind on each side, with a mouth between the front legs, and a few short hairs at the tail end (Fig. 71).

FIG. 71.—Sarcoptes Hominis (Female).

CHAPTER VIII.

EXAMINATION OF SEROUS EXUDATIONS, CEREBRO-SPINAL AND CYSTIC FLUIDS.

Serous Fluids from the Peritoneal or Pleural Cavities.—These are alkaline in reaction, contain 1 per cent. of salts, are usually of a straw-yellow colour, and either quite clear or slightly turbid. The important points to examine are:

The Specific Gravity.—Take this in a specimen-glass with an ordinary urinometer. It varies from 1005 to 1035.

The Amount of Albumin.—This may be estimated by Esbach's albuminometer (p. 40). Since readings above 5 parts per 1,000 are inaccurate with this apparatus, the fluid must be diluted with water once, twice, thrice, four times or five times, as may be necessary, the albumin in the diluted fluid estimated, and the reading multiplied by 1, 2, 3, 4, or 5, as the case may be, to give the parts per 1,000 in the original fluid. It varies from 2 to 40, but more commonly lies between 10 and 30.

Importance.—From the specific gravity of the fluid and the amount of albumin it contains it is sometimes possible to decide whether an effusion

be inflammatory—*e.g.*, tuberculous—or whether it be a passive dropsy, such as occurs with cardiac failure. In the former the specific gravity and the amount of albumin are higher than in the latter. But definite conclusions can only be drawn when the figures are extreme; that is to say, a specific gravity of 1020 or more, or 30 parts per 1,000 of albumin, would indicate an inflammatory origin. A specific gravity of 1010 or less, or 5 parts per 1,000 of albumin, would favour a diagnosis of passive dropsy. Figures between these would be compatible with either. On repeated tappings, an increase in the specific gravity or in the amount of albumin may be evidence of additional inflammation.

The specific gravity is no measure of the amount of albumin present. Ascitic fluid of low specific gravity may contain much albumin.

The Presence or Absence of Blood.—This may be looked for by the guaiacum test (p. 20), or by the microscope or spectroscope (p. 20).

Importance.—It is common to find blood in the later portions of a tapping, or in a serous effusion that has been tapped before. But if the fluid contain blood at the first tapping, it either indicates a very acute inflammation, or rouses a suspicion of malignant disease.

Cell Elements.—These are best examined by allowing the fluid to stand an hour or more in a specimen-glass, pipetting off and centrifugalizing the lowest part, and preparing and staining a cover-slip film of the deposit. This should then be examined under $\frac{1}{6}$ inch objective.

There may be no cells at all, or there may be leucocytes, squamous pleural or peritoneal cells, red corpuscles, or even particles of malignant growth. It is rare to find bacteria, owing to the great bulk of fluid in which they are suspended.

> *Importance.*—Presence of many cells, particularly leucocytes, is almost proof of the inflammatory origin of the effusion. It is said that further deductions can be drawn—for example, that presence of many lymphocytes indicates tubercle. This is not yet certain, and the main importance of looking for cells is to discover if the effusion be due to active inflammation or not.

Coagulation.—This may occur in both inflammatory and in passive effusions on standing. Should the jelly-like coagulum be large and appear quickly, the fluid is probably inflammatory, but otherwise no deduction can be drawn.

Presence of Fat Globules.—In rare cases the fluid is almost like milk, and the microscope reveals droplets which stain black with osmic acid. When the fluid comes from the peritoneum the condition is termed *chylous ascites*, of which one chief cause is infection with filaria. The blood should be examined for the embryos of this parasite (p. 78). In other cases it results from rupture of the receptaculum chyli after abdominal injury or obstruction to the thoracic duct. In the chest chylous effusion is very rare, but has occurred after injury to the thoracic duct in the neck.

CEREBRO-SPINAL FLUID.—This is clear, colourless, alkaline; specific gravity, 1002. It contains 1 per cent. of salts, a mere trace of albumin, and a body—pyro-

catechin—which reduces Fehling's solution on boiling, but does not ferment with yeast.

Importance.—After head injury, escape from the ear or nose of a clear fluid which reduces Fehling's solution is strong evidence that the base of the skull has been fractured.

PANCREATIC CYST FLUID.—This may be either clear or turbid. It is of watery consistence, alkaline, with 1 per cent. of salts, a specific gravity of about 1005, and little or no coagulable proteid. The essential characteristic is that it contains proteolytic and amylolytic ferments. These may be tested for as follows:

1. *Proteolytic Ferment.*—Put a disc of hard-boiled white of egg into a test-tube, add some of the fluid, and stand in a water-bath at 37° C. for an hour. Should pancreatic ferment be present, the egg-white will have partially dissolved in the alkaline medium.

2. *Amylolytic Ferment.*—Boil some water in a test-tube, and carefully stir in some finely-powdered starch until a thin mucilage is obtained. Cool to 37° C. and add some of the fluid. From time to time take a drop of the liquid and test it with a drop of iodine. If amylolytic ferment be present, the starch will rapidly cease to go blue with iodine, but will presently go red instead, and finally will remain colourless, from the formation, in the first instance, of erythrodextrin, and later of achroödextrin. The ferment will further convert the latter into maltose and dextrose, which may be detected by their reduction of Fehling's solution.

Importance.—Pancreatic cysts occur from time to time. The fluid is not usually obtained until a

laparotomy is performed. The diagnosis may be obscure, for the affection may be simulated by retroperitoneal and hydatid cysts, or by local collections of ascitic fluid shut off by adhesions. It is by the above examination that the true nature of the cyst can be decided.

Hydatid Cyst Fluid.—This is colourless, clear or opalescent, alkaline; specific gravity about 1010. It contains but a trace of albumin, and by this may be distinguished from ascitic fluid. The main diagnostic point, however, is the presence in it of the 'hooklets' (Fig. 72) of dead scolices. It must be borne in mind that a hydatid cyst may grow to a great size, with production of daughter and granddaughter cysts, and yet may never have produced scolices. The fluid from such a 'barren' cyst will contain no hooklets.

Fig. 72.—Hydatid Hooklets.

Hydrocele Fluid.—The characters are similar to those of ascitic fluid and almost as variable. It is important, however, to centrifugalize and examine with the microscope for spermatozoa (Fig. 15, p. 13). An ordinary hydrocele should contain none, nor should a hydrocele derived from any part of the funicular process. Sometimes cysts arise from aberrant tubules of the testis; these are called spermatoceles, to distinguish them from true hydroceles. The fluid in them contains spermatozoa.

Ovarian Cyst Fluid.—There are two types:

1. True ovarian cysts contain a viscous brownish-green fluid, which pours almost like treacle. It has

a high specific gravity, alkaline reaction, and 1 per cent. of salts. Albumin is usually present in considerable quantity, and microscopical examination shows typical cylindrical epithelial cells.

2. Parovarian cysts contain watery, colourless fluid, alkaline, of low specific gravity, with 1 per cent. of salts. Albumin is scanty, and the few cells which may be found are not cylindrical but squamous.

Importance.—The nature of the fluid may be the only means of determining in what structure the cyst arose. The prognosis is more serious in the case of a true ovarian than in that of a parovarian cyst.

CHAPTER IX.

TESTS FOR THE COMMONER POISONS.

ARSENIC.

1. *Marsh's Test.*—Put a piece of pure zinc into a flask (Fig. 73, B), cork, and through the thistle funnel C pour a little distilled water. Add pure sulphuric acid until there is brisk evolution of hydrogen. Collect a little of the gas escaping from the side-tube A in a test-tube, and ignite it; if it burn in the test-tube without explosion, all the air has been expelled from B. Ignite the hydrogen jet A. Hold a cold porcelain dish in the flame. No stain should be deposited on the white surface if the hydrogen be pure. Now pour in some of the suspected stomach contents through C, and presently hold the cold porcelain dish in the flame A again. If arsenic be present it will be coming off as arseniuretted hydrogen, and will be deposited as a shining black metallic stain on the white dish.

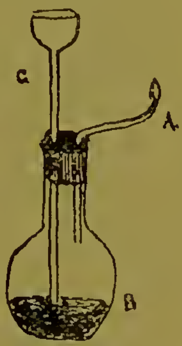

FIG. 73.

Antimony will give a similar black stain, but it lacks the metallic lustre.

2. *Reinsch's Test.*—Strongly acidify the stomach con-

tents with hydrochloric acid, and allow to stand one hour. Filter. Introduce into the filtrate a brightly polished strip of copper foil, and boil. If arsenic be present, a steel-gray coating will appear upon the copper; this may be further tested by dissolving in nitric acid, and evaporating to dryness. On adding silver nitrate to the residue, a brick-red precipitate of arsenate of silver will appear.

The difficulty of the test is to insure both the acid and the copper being free from arsenic to begin with.

Carbolic Acid.

Just acidify the gastric contents with hydrochloric acid. Filter. To the filtrate add bromine water. Carbolic acid gives a dense yellow precipitate of tribromophenol.

Oxalic Acid.

Boil the gastric contents with caustic potash. Even if lime had already been given to the patient, potassium oxalate will be formed. Filter. To the filtrate add solution of calcium sulphate; oxalic acid will give a dense white precipitate, insoluble in acetic, but soluble in hydrochloric, acid.

Hydrochloric Acid.

Filter the acid stomach contents. Add nitric acid to the filtrate, and then silver nitrate solution. A dense white precipitate of silver chloride results. Normal gastric juice contains hydrochloric acid, which will give the same test; a control must be made with

a 0·2 per cent. hydrochloric acid solution, and the density of the two precipitates compared.

Nitric Acid.

Neutralize the gastric contents with carbonate of potash. Filter. Evaporate the filtrate to small bulk. Mix with an equal bulk of strong sulphuric acid, allow to cool *completely*. Pour into a test-tube, and carefully add a solution of ferrous sulphate. The latter fluid floats upon the former, and the appearance of a black ring at the junction of the two indicates nitric acid.

Sulphuric Acid.

Filter the acid stomach contents. Add a solution of barium chloride to the filtrate. Sulphuric acid gives a dense white precipitate of barium sulphate, insoluble in hydrochloric or nitric acids. The test would naturally be valueless had any sulphate such as that of zinc been given by the mouth.

Corrosive Sublimate.

Mix the gastric contents with an equal bulk of ether, and shake up well. The ether will dissolve the corrosive sublimate. Pour off the ethereal solution; concentrate it by evaporation; add iodide of potassium. Mercury will give a scarlet precipitate of iodide of mercury, soluble in excess of potassium iodide.

Phosphorus.

Dilute the vomit with twice its bulk of water, acidify with sulphuric acid, transfer to a glass retort with a long condensing tube, and distil in the dark. Minute

traces of phosphorus will render the condensing vapour luminous.

Prussic Acid.

This may be evident by the smell. To test for it, exactly neutralize the gastric contents whether they be acid or alkaline, transfer them to a flask, and distil slowly upon a water-bath. To the distillate add silver nitrate; prussic acid will give a curdy white precipitate, insoluble in cold nitric acid, but soluble in hot. To confirm add liquor potassæ and hydrochloric acid to the precipitate, and then a few drops of sulphate of iron solution. If prussic acid be present, Prussian blue will be formed.

Alkaloids.

Before testing for the following alkaloids they must be extracted from the gastric contents. One of the simpler processes for this is as follows:

Otto's Method.—Acidify the vomit with weak sulphuric acid to convert the alkaloid into the sulphate. Strain through muslin. Agitate the filtrate with ether to remove all fat. Pour off the ether; render the residual watery solution alkaline with potassium carbonate to liberate the alkaloid. Shake up with ether again; the sulphate of the alkaloid was insoluble in ether, but the alkaloid itself is soluble. Concentrate the ethereal solution, and test it as follows:

Belladonna.—Add a little fuming nitric acid; dry upon a water-bath; cool, and moisten with a drop of potash dissolved in absolute alcohol. Atropine will give a *violet* colour changing to red.

Opium.—Evaporate the ethereal solution to dryness; dissolve in cold strong sulphuric acid, and add a drop of strong solution of bichromate of potash. Morphia gives a bright *green* colour.

Strychnine.—Evaporate the ethereal solution to dryness; to the residue add 2 drops of sulphuric acid, and then 2 drops of strong bichromate of potash solution. Strychnine will give a beautiful *purple-blue* colour changing to crimson, and finally to a light red.

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THE END

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